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U.S. NATIONAL CANCER INSTITUTE

ANNUAL REPORT of PROGRAM ACTIVITIES

July 1, 1976 through September 30, 1977

Part III A

DIVISION OF CANCER CAUSE AND PREVENTION

U.S. NATIONAL CANCER INSTITUTE

Division of Cancer Cause and Prevention

ANNUAL REPORT of Program Activities.

July 1, 1976 through September 30, 1977

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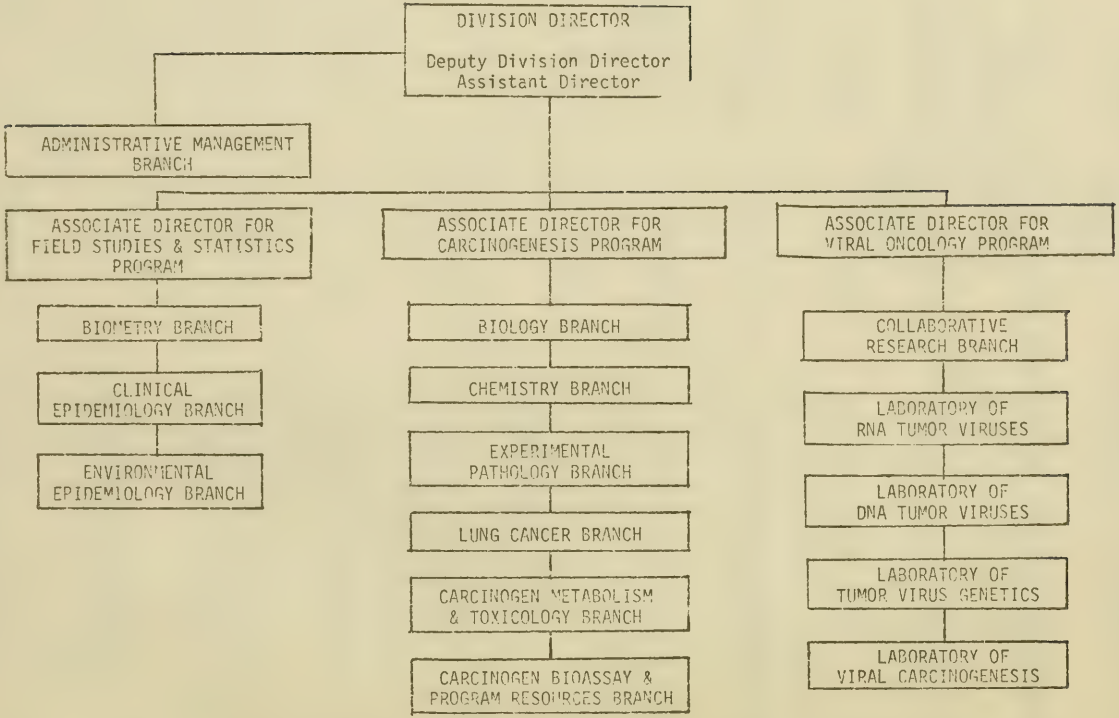
*S.C. - Service Contract

DCCP Organizational Chart



SUMMARY REPORT

DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE

July 1, 1976 - September 30, 1977
Dr. James A. Peters, Director

The summary report of the Director, Division of Cancer Cause and Prevention sets forth goals and objectives of the Division, a conceptual base upon which a broad attack on cancer etiology and prevention can be developed, objectives and program approaches to be used for their attainment, and the magnitude of the task and investments required.

A simplified synopsis of major goals, and of pathways to them, is given in Figure 1. More detailed program logic networks for Field Studies and Statistics, Carcinogenesis, Viral Oncology, Smoking and Health Program, Diet, Nutrition and Cancer Program, Office of the Scientific Coordinator for Environmental Cancer, and the Clearinghouse on Environmental Carcinogens will be found under specific area presentations.

Activities in DCCP are specifically directed towards cancer prevention. Research output is continually utilized in resetting more advanced priorities and in formulating those activities that offer the highest promise of success. The major structures of this scientific management process are indicated in Figure 2.

Newly enacted legislation and funding for the National Cancer Program pose novel challenges and opportunities in research and for cancer control activities in man. This legislation and program recognize the promising advances of cancer research which now make it possible to formulate direct intervention in man's behalf: the definition and engineering of a less hazardous environment and lifestyles, and the identification of susceptible individuals.

Scientific, public and legislative awareness of the role of environmental factors as cancer causes has increased considerably during the last year. This was particularly reflected in the establishment of the National Clearinghouse on Environmental Carcinogens within the Office of the Director, Division of Cancer Cause and Prevention. The activities of the Clearinghouse will be described in more detail later in this report.

IMPACT OF CANCER

Cancer occurs in all parts of the world, but variation in the incidence of specific forms of cancer is the rule rather than the exception. A range of ten or twenty-fold is common and for some types of cancer it is far wider.

The mean rate of cancer mortality in the United States occupies an intermediate rank in the worldwide range, but the rank of mortality from specific types of cancer varies markedly. The United States' white population has

the lowest mortality from cancer of the stomach and close to the highest from cancers of the colon and female breast. As shown in Table 1, the incidence rates of various cancers in the U.S. are expected to increase from a low of 25% each for leukemias and breast cancer to a possible high of 38% for cancer of the pancreas. This table also presents leads towards identification of etiologic factors and towards prevention.

Available evidence suggests that environmental agents and social practices, rather than conventional genetic factors, are largely responsible for variation in the occurrence of cancer in different populations. It should, therefore, be possible to reduce the occurrence of the major types of cancer in the United States to the level of the lowest ranking country for that type. Such a reduction would cut mortality from cancer by one third. This is the minimum goal we should aim for.

More than 52 million Americans now living will eventually have cancer (one in four persons) according to present rates. Cancer will strike over the years in approximately two of three families. In the 1970's in the United States alone, there will be an estimated 3.5 million cancer deaths, 6.5 million new cancer cases, and 10.0 million under medical care for cancer unless the present trends are moderated. Such trends mean that in 1977 there will be 385,000 cancer deaths and 700,000 new cases and in 2000, 471,000 cancer deaths and 1,085,000 new cases.

Table 1 also indicates the overwhelming causative effects of cigarette smoking and dietary habits: perhaps as much as two thirds of all cancers are related to these factors. Rapid progress is likely to take place in the control of smoking-related cancers through educational campaigns, the development of less hazardous cigarettes and the identification of individuals at risk. Dietary factors appear more complex; clear-cut solutions will need to wait until more fundamental research and questions are resolved; however prudent dietary recommendations can be made with available knowledge, without having to wait for the complete clarification of etiologic mechanisms. Occupational carcinogens are also being detected with increasing success; although it is estimated that less than 10% of all cancers may be of occupational origin, the identification of occupational risks usually leads to rapid and effective preventive measures.

Although cure rates for cancer patients have steadily improved over the past several years, the mortality (both in actual numbers and in rates corrected for population growth and other changes) continue to rise. This is because new cancer cases are appearing at an even greater rate than the improved rate of cures.

PROGRESS, PROBLEMS AND OPPORTUNITIES

A. Field Studies and Statistics

Cancer can be prevented by identifying its causes and removing them, and/or by increasing the body's defenses against them. Thus, it is important to know not only what causes cancer, but also who is unduly susceptible or resistant. Susceptibility may be immense. For example, if an identical

twin develops leukemia during the first year of life, his co-twin is virtually certain to develop leukemia within weeks or months. This increased risk fades quickly with age, and entirely disappears by 6 years of age.

Recently there has been substantial increase in our list of characteristics of persons who are unusually susceptible to leukemia, lymphoma and other cancers. Once such a list has been created, it can be examined for a common denominator. Many items can be explained by a known or possible genetic defect, sometimes extensive enough to cause various abnormalities in the appearance of chromosomes. It should be noted that the only environmental agent known to be leukemogenic in man, ionizing radiation, and the strongest suspect among the chemicals, benzene, produce long-lasting chromosomal abnormalities in somatic cells.

By contrast, persons at high risk of lymphoma (solid tumors of lymph tissue) have inborn immunological deficiencies (ataxia-telangiectasia, Wiskott-Aldrich syndrome, or congenital x-linked agammaglobulinemai). Consistent with these observations is the increase reported in the occurrence of lymphomas among persons given immuno-suppressive drugs following renal transplantation. A sudden upturn in one from of lymphoma, Hodgkin's disease, at about 8 years of age, also suggests a relationship to immunological development. Normally, at this age, there is a marked spontaneous involution of lymphoid tissues throughout the child's body. Lymphoid tissues are centers of immunologic activity. It is tempting to think that some failure in this normal regressing accounts for the upturn in the occurrence of the neoplasm.

There are many other inborn defects that carry high risk of cancer of various types. In the aggregate, they do not account for a large proportion of cancer but they probably do constitute a large percentage of cancers among young people. In this group, prevention or early detection and treatment can provide decades of healthy life. Peaks soon after birth in the frequency of diagnosis of leukemia, neuroblastoma, Wilms' tumor, and certain forms of brain cancer indicate that many of these neoplasms are likely to have been initiated during the intra-uterine life.

Knowledge of these relationships permits physicians to examine children with certain defects more closely than usual, since early detection and prompt removal of these tumors can be life-saving. Scientists, on the other hand, can explore the origins of the tumors in the light of what is known of the associated birth defects. Thus, instead of studying a tumor in terms only of itself, new avenues of approach have been opened. What is learned through these rare mistakes of nature may well apply to prevention of large categories of cancer. In particular, the epidemiologic identification of individuals and populations at increased risk of different cancer patterns aids the investigator in virology and chemical carcinogenesis in searching for specific etiological factors.

International Activities

A contract with NAS-NRC supports and extends cancer research at the Atomic Bomb Casualty Commission in Japan. This has paid off in the modified Japanese attitude toward ABCC, so that there is now substantial involvement

of several Japanese universities, including the development of a tissue registry at Hiroshima. These resources provide on-the-ground data resources for the development of epidemiologists. The field studies with the International Agency for aesearch on Cancer (IARC) in Iran on cancer of the esophagus have progressed very well.

Under the aegis of the UICC, the Epidemiology Branch is collecting data for an international comparison of pediatric cancer around the world. A monthly newsletter is used to communicate important observations on the origins of childhood cancer to pediatric oncologists throughout the world.

The international registry of epidemiology and epidemiologists (as a part of the International Cancer Research Data Bank) is about to being operation as a cooperative activity of UICC and NCI.

Work has continued on exploiting special populations in India (the Bombay Cancer Registry) and in Cali, Colombia (problems of cervical cancer, Hodgkin's disease). Migrant studies of Norwegian, Polish and Japanese migrants to the United States are being continued - although at a relatively low financial level.

The cooperative work with Soviet Russia in cancer epidemiology is continuing and has been expanded through exchange of information on data on mortality incidence and survival in the two countries.

Domestic Activities

Two major thrusts define domestic studies: data gathering and analysis, and the testing of etiologic hypotheses. With respect to the former, the report of the Third National Cancer Survey (TNCS) was published, for all three years of the survey, and by survey area. Also cancer mortality data, by county, by site, by sex and race for the 20-year period 1950-1969 were published, giving total numbers of cases, and rates. This will be followed by maps, permitting visual identification of clusters. Hospital cost data have been gathered by TNCS. Information on treatments and survival has been reported in the 4th End Results Report. The Third National Cancer Survey and the End Results Program have given way to SEER (Surveillance, epidemiology and End Results) Program. Through this program, we hope to develop early data on incidence, mortality and survival, on a grass-roots level, hopefully to provide contemporary data to guide the search for etiologic factors and other control activities.

A special interest in familial and genetic cancers and in childhood cancers continues. Familial cancers (e.g., medullary carcinoma of the thyroid and pheochromocytoma) give an opportunity for effective secondary prevention through frequent examination of family members at high risk. Childhood cancers are being pursued through a national registry of death certificates, a followup of children born in military hospitals where data exist on their intra-uterine exposure to x-irradiation, and in cooperation with the Third National Cancer Survey, will permit study of incidence as well as mortality.

The National Wilm's Tumor Survey has confirmed the high rates of aniridia, and hemihypertrophy in Wilm's tumor children. These are in addition to previously observed high frequency of genito-urinary defects and familial incidence.

Other major areas of work involve studies of prognostic factors, particularly in breast cancer, and Hodgkin's disease. Am emphasis on the characteristics of patients who may or may not benefit from certain forms of therapies was developed out of the cooperative studies of cancer of the prostate, undertaken with the Veterans Administration. These studies helped tailor treatments to prostate cancer patients. The concept of the "Epidemiology of Treatment" arose out of these studies and it should develop into a fruitful new field of study.

B. Carcinogenesis

The observed relation between exposure to certain chemicals and the development of cancer in man has been historically the first step towards knowledge of the etiology of cancer. A listing of agents persently known to produce cancers in man includes some 10 specific chemicals, 12 mixtures and crude products and, in addition, radioactive materials and radiation (including x-rays and ultraviolet light). The number of items is relatively small, because studies capable of identifying an agent as directly causative of cancer in man require large-scale and time consuming epidemiologic studies and have been conducted for a very small proportion of the potential exposures of man. In fact, essentially all materials that have been demonstrated to be carcinogenic for man have also been found to be carcinogenic in animals.

Most chemical carcinogens known from animal studies appear active in a variety of animal species. It is estimated that more than 1000 chemicals have so far been found carcinogenic in animal bioassays out of about 6000 that have been tested.

The major types of cancer in man that can be directly related to known carcinogenic exposures are as follows: a) cancer of the lung related to cigarette smoke and certain other inhalation hazards (particularly asbestos, chromates, and radioactive materials); b) cancer of the skin related to exposure to a variety of crude tar products and combustion products as well as to ultraviolet light and other forms of radiation; c) cancer of the bladder related to exposure to aromatic amines and their derivatives; d) leukemias related to exposure to radioactive materials; and e) mesotheliomas related to asbestos exposure. A number of other human cancers have been considered to be related to exposure to environmental carcinogens but the evidence is less clear.

Because of the widespread use of some of these materials, the proportion of the total population exposed and affected is extremely difficult to estimate, with the exception of such materials as cyclamates, DDT and tobacco smoke. It has been estimated that the majority of the present cases of lung cancer in the United States are attributable to cigarette smoking and that factor alone appears to be responsible for a death toll in the neighborhood of 60,000 deaths a year.

The development of our knowledge on the causative factors of cancer in man can be projected along the following lines: a) epidemiologic studies to correlate specific exposures to certain types of cancer in man with particular emphasis on occupational exposures; b) additional bioassays in animal models which will identify chemicals as carcinogenic in animals and therefore potentially carcinogenic in man; c) studies of animal human correlations by comparisons of bioassay results with the results of epidemiologic studies or by bioassays on isolated human tissues by in vitro techniques; and d) by studies of pharmacologic and metabolic correlations among species.

The major areas of opportunity and need for progress in cancer prevention through studies in Carcinogenesis are: a) develop better means for the removal of hazardous chemicals from the environment; b) develop means to enhance the ability of the host to detoxify environmental agents; c) determine the extent to which chemicals act in synergistic concert with viruses or with physical carcinogens such as irradiation; and d) develop more rapid and more sensitive means to detect and bioassay the cancer-inducing effects of chemicals for man. The latter area of activity is particularly important. For example, it is estimated that approximately 2,000 new chemicals enter our environment to a significant degree each year. With present assays it takes over 3 years and 400 animals and over $100,000 to test one chemical. It is hoped that newer studies in animal and human tissue cultures will diminish these requirements. However, until this is achieved the bioassay resources are limited to a few hundred compounds annually and it is necessary to select compounds to be tested on the basis of their distribution in the environment, intake in man and similarity to known carcinogens.

Program Highlights

1) Bioassay. A contract at the Stanford Research Institute, directed at establishing a ranking of suspicion of carcinogenic hazard for compounds present in man's environment, provides information for the best utilization of current limited bioassay resources and may give early guidelines to the chemical industry, as to the hazards that may be expected by the introduction of a new compound.

The significance of current bioassays for carcinogenicity derives mainly from two considerations, namely, that, a) humans in general react like other animals to a variety of insults, the differences being usually quantitative rather than qualitative and, b) current bioassay methods, even when performed with adequate biometrical and qualitative controls, can be expected to detect only those compounds that are strongly carcinogenic.

Then two reasons prescribe that the greatest attention be given to positive results in current carcinogenicity tests.

Testing and retesting of environmental chemical pollutants at NCI continue to include agricultural chemicals such as pesticides, artificial sweetners such as cyclamates and saccharin, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Food preservatives are also under constant scrutiny.

A bioassay prime contractor, selected to perform in a comprehensive way the many tasks involved in this area, has enhanced the bioassay capabilities.

Because of the need to involve a larger segment of the scientific community and of society at large in the proceedings of the bioassay surveys, the National Cancer Advisory Board has recommended and the National Cancer Program has established a Clearinghouse on Environmental Carcinogens. The Clearinghouse advises the National Cancer Program in all aspects of the bioassay surveys; selection of compounds; definition of testing protocols; the evaluation of test data; assessment of risk. Membership in the Clearinghouse is from the academic world, industry, labor, and consumer groups. Governmental agencies are ex officio participants. The Clearinghouse has been operational since the end of June 1976.

2) Development of Bioassay Models. Major programs have been established for the development of biological models for tumor pathogenesis closely correlated to the main types of cancers in man. Lung Cancer and Colon Cancer Programs are well established; Pancreas Cancer and Prostate Cancer programs, initiated in FY 1972, are now developing programs on Endocrine Cancers and childhood cancers. Their emphasis is on the use of animal models and human material to identify pathogenetic steps susceptible of inhibition or prevention.

In vitro models for neoplastic transformation of cells in culture by chemicals have been developed using cells from hamsters, mice, and guinea pigs. A major advance was the development of a host mediated transformation assay, responsive to carcinogens that require metabolic activation by the host. These new methods are being used to establish their value both as screening techniques and as tools for identifying key steps in carcinogen activation and interaction, susceptible to inhibition. The applicability of these techniques to transformation of human cells is currently being investigated. Genetic factors and growth conditioning factors required for neoplastic transformation by chemicals have been identified. Enhancement of chemical cell transformation by radiation and of viral transformation by chemicals have been demonstrated. Transformation of epithelial cells in vitro by chemicals has also been obtained in preliminary experiments. A major advance has been made with the transformation of human cells in vitro by chemical carcinogens, conditioned by corticosteroids.

Because of the significance of in vitro bioassay to toxicological programs in general, and major involvement of several Government agencies on this topic, the In Vitro Program has been located directly in the Office of the Director, DCCP, where all the efforts for coordination with other Federal agencies have been centralized for the last two years.

3) Studies of Carcinogenesis Processes. New emphasis has been given to the study of carcinogen metabolism and toxicology, and a large collaborative effort is under way in the documentation of the conditions of formation and activation of nitrosamines. Pharmacologic inhibition of the formation of carcinogens has been obtained by blocking nitrosation of amines. Other classes of carcinogens are also under study (e.g., hydrazines, metals).

Major metabolic steps in the ensymatic activation of several classes of carcinogens have been identified with special emphasis on polynuclear hydrocarbons. Their reproducibility in human tissues has already been demonstrated in some cases. Micromethods, applicable to determination in small samples of human cells, have been developed for measuring carcinogen activation levels. Inhibitors of carcinogen activation have been identified. The development and use of high-pressure, liquid chromatography has been a milestone in the study of metabolism of polynuclear hydrocarbon carcinogens. This technique can separate as many as ten metabolites of benzo(a)pyrene in a short period of time and can therefore correlate carcinogen susceptibility with unique metabolic profiles.

4) Immunological Studies in Carcinogenesis. Radioimmunoassays have been developed for several carcinogens and related compounds. Progress was made in the study of the components of BCG responsible for tumor cell killing and in the study of the mechanism of action of cell-mediated immunity.

Refined methods have been developed for measurement of serum of carcino-embryonic antigen and rat alpha$_1$-fetoprotein.

C. Viral Oncology

It has become evident that cancer is the result of a complex interaction among many factors and identification of a virus alone may not be the complete answer to the cancer problem. A number of factors that interact with viruses to control expression have already been identified and they include both host factors such as genetics, hormones, immune responses and environmental factors such as chemicals, x-rays, UV light, etc. The Program is therefore expanding and giving special emphasis to the study of all aspects of the control of virus expression.

The emphasis in cancer virology ahs moved from the search for viruses to the study of virus gene transcription, translation and control mechanisms. In the case of DNA viruses, which in contrast to RNA viruses can kill the cells they infect, defectiveness in replication appears to be a requirement for transformation. In all tumor viruses studies, the transforming information is contained in one gene or a small number of genes that can be expressed in the absence of other viral functions. It appears therefore that one gene, producing one product, can specifically switch a target cell to malignancy. The identification of the product of this gene and the study of its mechanism of action is a major thrust of tumor virus research at this time.

Viruses have proved useful in yet another context. Quantitative assay procedures involving viruses have been developed for the screening of potential chemical carcinogens.

RNA Viruses

RNA viruses have now been shown to cause a variety of malignant diseases in a large number of animal species ranging from birds to primates. Although infectious viruses have not been found in all animals, viral

markers are present in each case, including man, where they have been looked for with appropriate probes. It appears that RNA viruses are a normal part of the cells of all vertebrates and it is likely that they function in embryonic development and only under certain conditions do these viruses become involved in oncogenesis.

These highly oncogenic viruses contain two types of genes; those that are involved with viral replication and virion structural proteins, and those that are specifically responsible for transformation. Recently, it has become possible to separate the transformation gene sequences from the replication gene sequences. Essentially this is done by breaking DNA transcripts of viral RNA into pieces and adsorbing out those pieces that have replication sequences by a series of nucleic acid hybridizations, leaving only the transformation sequences.

The transforming sequences studies so far are rodent and avain sequences. It will be important to isolate similar genetic sequences from highly oncogenic primate viruses because only those can be expected to be sufficiently similar to human transforming genes to be used as probes for the presence of such sequences in man.

Now that the RNA viral genes responsible for transformation have been isolated, it is feasible to develop probes that will detect the transforming protein and to study its distribution, characteristics and mechanism of action.

Cancer Virology has moved into a new era; although we are still studying viruses we are now dealing with genes and their products and not necessarily with infectious viral particles. Finding an infectious human cancer virus is no longer necessary to study the role of viral genes in human oncogenesis.

Hormones also play an important role in virus expression. This has been clearly demonstrated for the type B RNA viruses, the growth of which is greatly stimulated by dexamethasone. Mammary epithelial cells, the target cells in which type B viruses grow, also are subject to hormonal control. This may reflect a significant relationship between cell activity and viral growth.

As has already been discussed, chemicals can affect virus expression. Indeed, the use of chemical and physical agents is a standard procedure for the activation of latent viruses; by such means many endogenous viruses were discovered.

DNA Viruses

The role of DNA viruses as etiologic agents of proliferative cellular diseases in several animal species is now well accepted. Early reports describing the capacity of certain murine and simian papovaviruses to induce malignant tumors in animals and transform cells in tissue culture provided the impetus for examining the relationship between various human DNA viruses and cancer in man.

The papovaviruses offer a unique approach for studying the phenomenon of transformation of a cell from the normal to the malignant state. These small, relatively simple viruses appear to perform the convenient function of packaging all of the necessary genetic information required for the induction of cancer. The fact that the DNA of these viruses can code for less than ten proteins makes them prime candidates for defining those virus-specified functions which are responsible for oncogenesis. Indeed, significant progress has already been made along these lines.

A key problem encountered in proving an etiologic role in cancer for those viruses has been their wide distribution in human populations throughout the world. Tumor formation is usually marked by an increased, rather than a de novo, immunological response to the virus. Antibody titers in cancer patients are statistically much higher than those background levels measured in carefully matched normal subjects. The question of why some individuals infected with these viruses develop cancer while others do not remains unanswered.

D. Smoking and Health Program

The overall objective of the Smoking and Health Program is to reduce the moribidity and mortality of smoking-related disease. This requires dual public action to:

- Promote public awareness of smoking hazards.
- Reduce the risk to those who continue to smoke.

The first of these actions has been undertaken through public education campaigns sponsored by the National Clearinghouse for Smoking and Health (now part of the Center for Disease Control, Department of Health, Education and Welfare), the American Cancer Society, the American Heart Association, and other private organizations. The efforts of these groups are continuing and include programs for special segments of the population, e.g., pregnant women and school-age children.

The National Cancer Institute's Smoking and Health Program has undertaken the second set of actions. In collaboration with the National Heart, Lung, and Blood Institute and the U.S. Department of Agriculture, the National Cancer Institute has addressed itself specifically to the problems of 1) identifying the individuals most likely to develop smoking-related diseases, 2) developing means fo reducing the risks of such individuals, and 3) designing less hazardous cigarettes.

Current Activities and Accomplishments

Experimental modification of cigarettes has taken place since 1950. Certain consistent results led to reasoned predictions of the effects of certain modifications on tumorigenicity. In 1970, under the direction of the Tobacco Working Group, researchers defined the first series of experimental cigarette blends, using these early findings as a basis.

Series II, initiated in 1972, applied the preliminary results of Series I bioassay and incorporated more agronomic factors. Variables of particular concern in this series included changes in amounts of tobacco per cigarette and aeration of the burning zone, use of tobacco from plants of various nicotine levels, use of various types and amounts of nitrogen fertilizer and suckering chemicals, use of tobacco with different leaf processing, and use of some artificial tobacco substitutes.

Based on the completed analyses of Series I and preliminary results from Seried II, the variables to be tested in Series II and IV were defined by the Tobacco Working Group. Series III cigarettes included more extensive manipulation of paper porosity and filters and changes in concentrations of sugars, humectants, and flavorings. Also included were proprietary artificial tobacco substitutes.

Series IV extended the accomplishments of Series II. Through the use of extracted tobacco and the addition of SEB having specified nicotine levels, researchers were able to manipulate the nicotine level of cigarettes more closely. Other variables of concern were new processes of reconstituted tobacco sheet with and without additives and the effects of waxes.

A fifth series is now in the planning stage. It is based on the "minimun cigarette concept"--maximum simplication of tobacco smoke through new applications of established tobacco processing techniques (i.e., reconstituted sheet technology, tobacco extraction, and tobacco dilution with inert fillers). This series will be designed primarily for inhalation bioassays rather than large-scale mouse dermal bioassays.

In addition to the above chemical analyses, a computer simulation model has been designed to aid in predicting design parameters of potentially less hazardous cigarettes. The simulation model also provides a rational description of the pyrolytic kinetics, thus enabling determination of the probable smoke composition of candidate less hazardous cigarettes. Simulation models can provide a link between design parameters and the elimination of undesirable smoke components.

Metabolic pathways of particularly significant smoke constituents have been investigated, with emphasis on nicotine, HCN, carbon monoxide, and oxides of nitrogen. Several techniques have been devised to obtain pharmacokinetic and metabolic data on these components. Organ deposition of radioactive HCN has been determined with rats. Other metabolic products have been traced after inhalation, and the effect on biological processes has been noted.

There is little doubt that through such research a less hazardous cigarette can be designed. It is important, however, that steps be taken to ensure smoker acceptability of such a product. An effort is being made to determine the organoleptic properties associated with a series of experimental cigarettes.

Bioassay for Tumorigenic Activity

The Smoking and Health Program screens the series of experimental cigarettes described above by means of a stepwise bioassay procedure.

A trend of decreasing tumorigenicity has been found for these series, indicating that cigarettes made with high-porosity paper, tobacco stems only, and reconstituted tobacco sheet are less tumorigenic. Thus, advances have been made in designing candidate less hazardous cigarettes.

Chemical/physical parameters have been statistically analyzed against tumorigenic potency, as evidenced in skin painting experiments. Analyses of Series I, II, and III have been published.

The second step in the current bioassay sequence is to test a selection of experimental cigarettes exhibiting low tumorigenic potency in mouse dermal bioassays, with whole-smoke inhalation models. Using automatic smoking machines, researchers have initiated the routine, chronic exposure of rats and dogs. Techniques have been developed to measure several parameters of cardiovascular and pulmonary function. Chronic exposure effects for animal models will be determined on the basis of the results of these tests and on the basis of histopathological data.

Dog inhalation models are being used to study the apparent correlation between nicotine content and tumorigenicity of experimental cigarettes, as found in mouse dermal bioassays. In another study, animals are being exposed for three years to obtain support for the histopathological data and to augment information concerning the effects of nicotine on cardiopulmonary systems. Extensive pulmonary function tests are being performed in a chronic smoke exposure study with dogs. It is too early to discuss the results of these studies; however, the data are expected to increase our knowledge regarding the extent of functional impairment attributable to cigarette smoking and to nicotine in particular.

In addition to studying pulmonary impairment, the effects of smoking on cardiovascular disease are being investigated. A 2-year study with dogs is being conducted to determine whether nicotine, carbon monoxide, and nitrogenous oxides accelerate the development of atherosclerosis. Another animal model, the pigeon, is being used to further document the effect of nicotine and carbon monoxide on atherogenesis.

Analysis of the experimental results indicates that cigarette materials can be manufactured to yield smoke with very low tumorigenic activity. The properties that have been found to be important in the development of less hazardous cigarettes are:

- Use of high-porosity paper as wrappers
- Large proportion of reconstituted tobacco sheet in the blend
- Use of tobacco extracted with water, hexane, or detergent
- Use of inert fillers as tobacco extenders
- Use of tobacco varieties from selected culture and cure methods
- Reduced tar content
- Use of filters and smoke dilution devices
- Use of flavor additives to ensure acceptability

Spurred by the activities and findings of the Smoking and Health Program, a number of less hazardous cigarette brands have appeared on the U.S. and international markets since September 1976.

Individual Risk Analysis

To ensure complete achievement of Smoking and Health Program objectives, it is desirable to identify those individuals at high risk of developing tobacco-related diseases. Epidemiologic studies are presently investigating the relationship between tobacco-related diseases and smoking habits.

The extensive data base for the United States now covers eight cities and is still expanding. Further data concerning specific smoking habits, types of tobacco smoked, etc., are being gathered.

In addition, epidemiologic studies have been initiated in five Western European countries. Because cancer incidence and mortality rates in Europe are different from thsoe in the United States, data on the relationship between smoking and cancer in these countries should increase present knowledge of cancer etiology. Cross-national comparisons of smoking behavior and cigarette types will be made with the American data. Knowledge of the causative factors involved in smoking-related diseases will facilitate the reduction of both incidence and mortality rates.

To gain additional knowledge regarding the smoking patterns of humans, attempts are being made to design a machine for investigating human dosimetry under normal smoking conditions.

Methods were also studied for the routine testing of susceptibility to tobacco-related diseases. One such test assays lymphocytes for carcinogen-induced aryl hydrocarbon hydroxylase (AHH) activity.

Because the multiple interactions of smoking and disease are responsible for nearly 500,000 premature death every year, the Smoking and Health Program offers a major opportunity in disease prevention. In particular, the demonstrated feasibility of less hazardous cigarettes should dramatically affect the epidemiology of the coming decades.

E. Diet, Nutrition and Cancer Program

In response to a mandate in the 1974 amendments to the National Cancer Act, the National Cancer Program developed the Diet, Nutrition and Cancer Program to develop and disseminate information on the interrelationships between diet and nutrition in the etiology of cancer and the therapy and subsequent rehabilitation of the cancer patient.

It is apparent that many chronic diseases such as heart disease, cancer, diabetes, arthritis, and dental caries are directly related to diet and nutrition. Much of the evidence comes from epidemiological investigations and some from laboratory animal studies and limited clinical observations.

Although there is no definitive scientific evidence of direct causation, there are various clues to indicate that the development of prudent, dietary habits early in life and the continuation of these habits throughout life may minimize the incidence of the above diseases.

Specifically, with respect to cancer, there is an increasing number of studies relating dietary and nutrient excesses, deficiencies, and imbalances to cancer development. For example, cancers of the stomach, colon, gastrointestinal tract, pancreas, and liver have been specifically correlated with certain dietary components.

Diet and nutrition are not only important in the etiology of cancer but they are also supportive procedures for the traditional modes of cancer therapy--surgery, radiotherapy, and chemotherapy. The research activities of the Program initiated in late 1976, and the first results will not be forthcoming until late 1977.

Etiology

This research element is oriented towards ascertaining the underlying causes and interrelationships among dietary and nutritional factors and cancer development. Information derived from etiological investigations will be utilized ultimately to develop prudent dietary recommendations to minimize the incidence of cancer and other diseases. Information will also provide the basis for developing new concepts for therapeutic regimens. Emphasis will be placed on ultimately defining desirable diets. Children will be a primary target for investigation because chronic diseases have relatively long latent periods and dietary habits are formed early in life.

Research in etiology will be conducted in the following areas:

Evolution - Examination will be made of the evolutionary determinants of dietary adaptation, of the natural dietary habits of man during his development, and animal models will be developed that might provide information and data on the effects of changing food habits.

Dietary Excesses and Deficiencies - This area includes acute and chronic studies of dietary alterations in man and animals to assess the effect of various dietary components on carcinogenesis.

Development of Methods for Assessing Nutritional Status - This area is oriented to the baseline compilation and analysis of information on dietary patterns and biochemical and metabolic parameters in various population groups.

Epidemiologic and Dietary Surveys - Leads will be developed using standard epidemiologic methodology in conjunction with dietary surveys using retrospective, prospective, and cross-sectional analyses.

Therapy

This research element is oriented towards the role of metabolic and dietary processes in the development of cancer and the use of dietary and nutritional supplementation in the support of traditional cancer treatment

modalities--surgery, chemotherapy, and radiotherapy. The possibility of utilizing diet and nutrition as a direct therapeutic regimen is also evaluated. Various methods of increasing the food intake of anoretic cancer patients will be investigated. The expected results to be derived from this element include the definition of dietary recommendations for optimal nutritional support, the development of suggested dietary intervention techniques to increase nutrient intake, the development of direct nutritional therapeutic solutions, and the development of cancer screening methods based on changes in metabolic processes. The overall improvement of the cancer patient's quality of life is the major goal of this research element. This will include assisting the cancer patient in coping with traditional therapeutic regimens, increasing the survival rates, and making rehabilitation easier. Emphasis will be placed on the pediatric patients because they have limited nutrient reserves for undergoing growth processes; from a psychological point of view, diet and proper nutrition could have a major impact on ability to cope with therapy.

The following research areas are included in the therapy element:

Host-tumor competition and food intake determinants: The objective of this area is to focus on changes in metabolic processes which are produced by cancer in man.

Anorexia: Studies will be undertaken on the alteration of taste and smell perception in the cancer patient and on the modification of food receptors and of food characteristics to improve caloric intake.

Artificial alimentation: The objectives of this research area are to demonstrate that those cancer patients who normally suffer from malnourishment can achieve a better quality of life when brought into a normal metabolic state with artificial alimentation, and to evaluate acute therapeutic results of current treatments in cancer patients who are in a normal metabolic state as a result of artificial alimentation.

Information

Besides the primary activity of assisting in the development and implementation of specific projects, the DNCP has also been involved in various information development and dissemination activities. These activities not only involve the preparation of documents for the scientific community but also for the lay public and the cancer patient. The program, with the assistance of the NCI International Cancer Research Data Bank, is presently in the process of developing the following program documents and services:

- State-of-the-Art Reviews: The objective of these documents is to provide the research community current information on the relationship of nutrient classes to cancer etiology and the use of nutrition in cancer therapy and rehabilitation.

- Cancergram: This current awareness service will help maintain scientific knowledge in rapidly changing disciplines.

. Cancerprojects: These documents would provide a listing of ongoing projects in both therapy and etiology for both the U.S. and foreign countries.

In addition, the DNCP has prepared two handbooks oriented to the parents of pediatric cancer patients and to adult cancer patients and their families, and dealing with specific dietary counseling.

Because of the manifold interactions of diet and nutrition with health, disease and therapy, the DNCP holds unusual promise of producing data of immediate application to man.

SUMMARY

Between 30% to 90% of cancer deaths in man are preventable. Mortality statistics from around the world show that if cancer mortality in the United States, site for site, was comparable with that of the lowest country reporting for each site, over 200,000 lives would be saved each year. The major sites in which gains should be made are cancer of the colon and the rectum, cancer of the lung, breast cancer, cervix and uterus (particularly among non-whites), cancer of the ovary, cancer of the prostate, and probably leukemia.

Information is already in hand to reach these goals for lung cancer and for cancer of the cervix and uterus. These two sites alone would account for 100,000 or more savable lives annually. A concerted research effort is called for in cancer of the lung, colon, breast cancer and cancer of the prostate. There is promise of substantial pay-off in these sites. For example, migrant studies have provided very strong evidence that cancer of the colon is a disease with a very strong environmental base, possibly related, among others, to high fat consumption. Japanese migrants to Hawaii show a three to five-fold increase in contrast to Japanese on the home islands.

Two hundred thousand lives is a minimum estimate. If we add to the preventable cases those that can be saved through earlier diagnosis and improved treatments, this number should be easily doubled. For example, if the most recent knowledge on early diagnosis of breast cancer were applied across the country, we might anticipate 12,000 fewer deaths each year from breast cancer alone.

Current work on identifying the cancer "susceptibles", if successful, will lead to cutting down cancer deaths. First, by identifying susceptibles, we will know what people to watch closely and regularly, so that we might diagnose their disease early, and thus treat it successfully. Second, by identifying the cancer susceptibles, it should be possible to protect them from those environmental assaults which could be carcinogenic to them - while essentially innocuous to others.

Ninety percent reduction in cancer deaths could be reached if, in addition to current knoweldge on prevention, and universal application of the best diagnosis and treatment techniques, our research uncovered the

interacting environmental elements and lifestyle habits which lead to different rates in different countries in the world; and the susceptibles whom we must and can protect.

Difficulties notwithstanding, opportunities for cancer prevention and control are very bright through application of existing knowledge and through a commitment to seek new information.

The most promising areas now developed are in the control of cigarettes smoking related cancers, in diet-related cancers, and cancers of occupational origin.

A Note on Management

The obligatory opportunity to plan and manage biomedical activities from basic or fundamental research upwards through development and application, with effectiveness and efficiency, requires skillful staff and line personnel of unabashed motivation, objectivity, and tact. Tact is of paramount importance because NCI management must persuade constitutent scientists to attack specific problems of human disease. We adhere to the belief that NIH exists primarily to allow people to live better and longer and that NCI abets this goal through the conduct of research and development for prevention and treatment of cancer in man. Tact, including the courage and reason to say no, is important also because, while the need for wide-spread program participation is high, many scientists do not understand that even successful completion of their proposed project may not necessarily contribute to the attainment of program goals. The thrust towards freedom of research must be tempered with appreciation for and selection of fundamental projects with expectation of eventual application to man. This can and should be done through both grants and contracts.

We are fortunate to have this admixture of talents and motivation in the Deputy Director, Dr. G. B. Gori, and Associate Directors, Drs. Moloney, Griesemer, and Schneiderman; the Assistant Director, Dr. W. G. Flamm, and the Assistant to the Director, Dr. R. Depue; and in the Administrative Officers, Mr. John Miller and Mr. Nicholas Olimpio. Particular recognition is entirely warranted to their associates, whose motivation to program goals exceeds their desire for personal recognition.

Please refer to summary and individual reports for details of activities within DCCP Programs for FY 1977.

CANCER ETIOLOGY AND PREVENTION PROGRAM

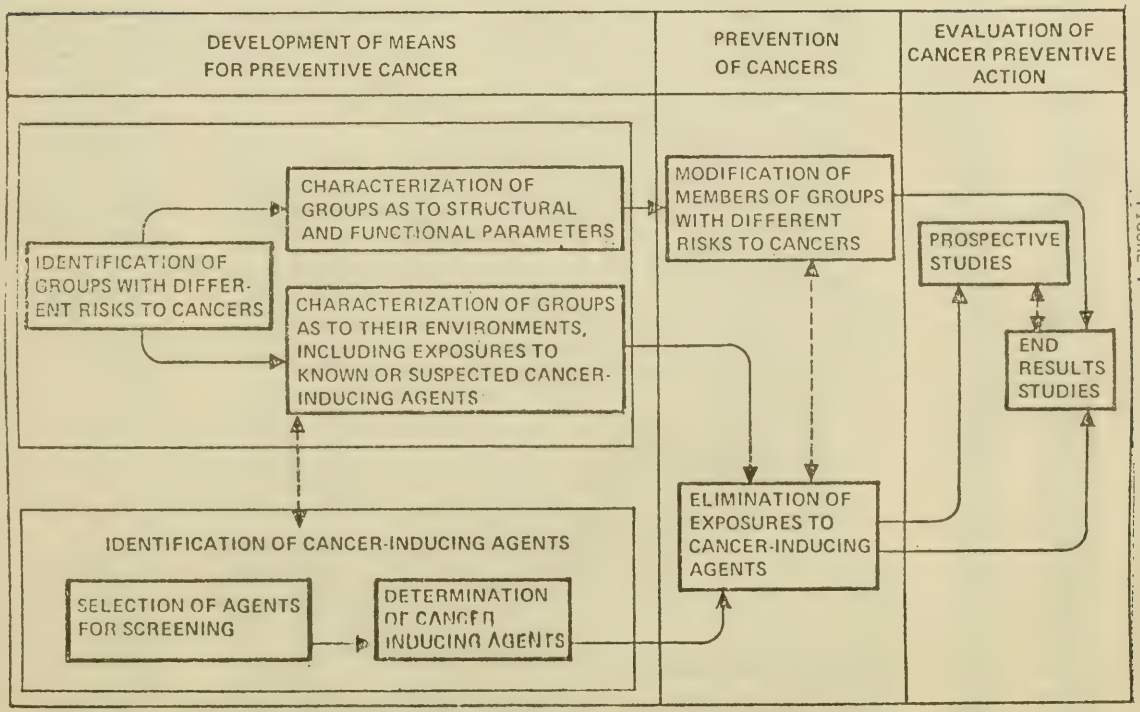

DEVELOPMENT OF MEANS FOR PREVENTIVE CANCER
PREVENTION OF CANCERS
EVALUATION OF CANCER PREVENTIVE ACTION
IDENTIFICATION OF GROUPS WITH DIFFERENT RISKS TO CANCERS
CHARACTERIZATION OF GROUPS AS TO STRUCTURAL AND FUNCTIONAL PARAMETERS
CHARACTERIZATION OF GROUPS AS TO THEIR ENVIRONMENTS, INCLUDING EXPOSURES TO KNOWN OR SUSPECTED CANCER-INDUCING AGENTS
IDENTIFICATION OF CANCER-INDUCING AGENTS
SELECTION OF AGENTS FOR SCREENING
DETERMINATION OF CANCER INDUCING AGENTS
MODIFICATION OF MEMBERS OF GROUPS WITH DIFFERENT RISKS TO CANCERS
ELIMINATION OF EXPOSURES TO CANCER-INDUCING AGENTS
PROSPECTIVE STUDIES
END RESULTS STUDIES

FIGURE 1

FIGURE 2

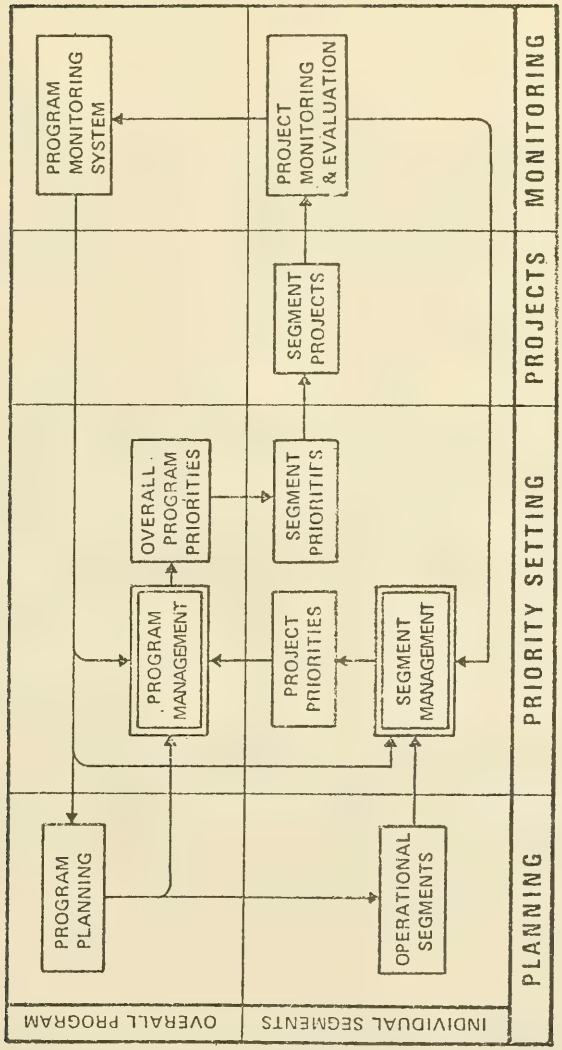

PROGRAM MONITORING SYSTEM
PROJECT MONITORING & EVALUATION
SEGMENT PROJECTS
OVERALL PROGRAM PRIORITIES
SEGMENT PRIORITIES
PROGRAM MANAGEMENT
PROJECT PRIORITIES
SEGMENT MANAGEMENT
PROGRAM PLANNING
OPERATIONAL SEGMENTS
OVERALL PROGRAM
INDIVIDUAL SEGMENTS
PLANNING
PRIORITY SETTING
PROJECTS
MONITORING

TABLE 1: CANCER INCIDENCE: EXPECTED NUMBERS, 1970-2000; CHANGE IN RATES, 1970-2000; DEATHS, 1970

Site	New Cases (Incidence)* 1970	2000	Deaths	Incidence Year 2000 % Change in Rate**	Major Causation	Means of Prevention
Lung	81,000	295,000	62,000	179	Tobacco Smoke Air Pollution (including on-the-job)	Stop smoking - Reduce pollution Less hazardous cigarette
Large and Small Bowel	92,000	134,000	44,000	11	Intestinal Flora? Heredity? Diet?	Viruses? Other insults. Identify susceptibles and eliminate their exposure.
Breast	82,000	111,000	30,000	4	Virus? Diet? (Hormones) Genetic?	Vaccines. Identify susceptibles.
Pancreas	20,000	35,000	18,000	38	Diet? Virus? Other Insults?	Identify etiology. Identify susceptibles.
Prostate	51,000	78,000	17,000	17	Hormones? Diet?	Identify etiology. Identify susceptibles.
Stomach	21,000	4,500	16,000	-84	Diet Poor socio-economic conditions	Diet modifications. Sociologic modifications?
Leukemias	20,000	32,000	15,000	26	Viruses. Radiation. Genetic.	Vaccines. Identify susceptibles and limit radiation.
Non-melanotic skin	376,000	585,000	5,000	20	Actinic rays. Genetic.	Limit radiation exposure. Identify susceptibles.
Misc.	242,000	408,000	75,000	30	Multiple	Identify extrinsic and intrinsic factors and modify them.

*Based on data from Third National Cancer Survey, 1969-1970. In situ cancers have been excluded.

**Projected change in age-adjusted incidence rates (year 2000 compared to 1970) assuming the trend in rates noted from 1947 to 1969 continues to the year 2000.

SUMMARY REPORT

SCIENTIFIC COORDINATOR FOR ENVIRONMENTAL CANCER

July 1, 1976 to September 30, 1977

Mission

The Scientific Coordinator for Environmental Cancer is located organizationally in the office of the Director of the Division of Cancer Cause and Prevention and serves as a focal point for development of program interests and collaboration in environmental cancer. This function involves working with other federal and state agencies, industrial and academic institutions, trade unions and consumer oriented groups. Close liaison with other agencies and institutions is maintained to insure proper emphasis and attention toward identifying potential environmental carcinogenic hazards for the general population and in workplace environments. These collaborative and coordinated efforts fulfill one of the essential requisites of the National Cancer Act and the mission of the National Cancer Program.

Activities of the Interagency Collaborative Group on Environmental Carcinogenesis, now in its fourth year, are directed and logistically supported through this office. The tasks taken on by this group are not strictly in the area of development and dissemination of data but indeed serve as a mechanism for achieving program collaboration on a day-to-day basis. The establishment of the Clearinghouse on Environmental Carcinogens has also extended the mission and added to the functions of this office, specifically in interagency and industrial activities, all related to the carcinogen testing program.

Other Assigned Functions

Because of the need to provide a broader overview and review process on environmental chemicals selected for carcinogenesis bioassay, this unit has been assigned the responsibility for the Chemical Selection Working Group on nomination of chemicals for bioassay. Also, the functions and activities have increased greatly due to the interfacing and logistical support provided in operation of the CCTRP (DHEW Committee to Coordinate Toxicology and Related Programs) Subcommittee on Carcinogenesis Testing and CEC (Clearinghouse on Environmental Carcinogens) Chemical Selection Group.

Accomplishments

The collaborative effort takes several forms; the first through the ICGEC (Interagency Collaborative Group on Environmental Carcinogenesis) which meets every six weeks. The second is through the day-to-day communication and cooperation with federal and industrial groups as the Division focal point on environmental cancer. The third activity is, as mentioned earlier,

through the work on Chemical Selection Working Group and Committee to Coordinate Toxicology and Related Programs/Subcommitee on Carcinogenesis Testing.

New groups join the ICGEC from time to time; it now has 58 members representing 28 agencies or subagencies. This year there were seven meetings making a total of thirty-four meetings since the formation of ICGEC in January 1973. This effort involves the primary interfacing with EPA with collateral interest and assistance from United States Department of Agriculture, Center for Disease Control, Food and Drug Administration, Energy, Research and Development Agency, United States Department of Interior (Fish and Wildlife Service) and National Institute of Environmental Health Sciences. The major objective is to publish a handbook and reference source on body burden of chemicals (carcinogens and noncarcinogens) to be used in support of CSWG, Office of Toxic Substance/EPA, and for epidemiological studies. Assistance was provided to EPA, NIOSH and FDA in the review of over 20 criteria and standards documents (EPA and NIOSH) and petitions (FDA).

Interfacing with Trade Associations, Industries and Labor Unions

The interfacing with trade associations and various industrial components has been greatly intensified during this past year. A major impact has developed in the Division's ability to respond to industrial requests for guidance and assistance in construction of facilities, animal model selection, protocol development and the mutually profitable exchange of information and data. These types of activities have become major functions of this unit. We met with over 20 organizations, including seven foreign, during the year.

Continuing dialogue has developed with industrial representatives concerning parallel studies (bioassay program and industrial consortiums) of several chemicals, e.g., methylene chloride and 1,3-butadiene, that are of vital importance to a very wide spectrum of industries, associations and federal regulatory agencies. The free exchange of protocols, preliminary phase results, etc., serves to enhance the studies of all and leads to the conservation of strained national resources, i.e., veterinary pathologists, toxicologists, and suitable animal testing space.

Information Exchange and Communications

This unit has served as a focal point for many inquires on environmental cancer from many sources. During this year we received over 400 requests. Our volume of mail has indicated that our number of communications has risen from 1600 in fiscal year 1976 to 2300 in fiscal year 1977. In addition, the industry liaison office has handled approximately 200 information requests.

SUMMARY REPORT

CLEARINGHOUSE ON ENVIRONMENTAL CARCINOGENS

July 1, 1976 - September 30, 1977

The Clearinghouse on Environmental Carcinogens was chartered May 5, 1976. Its primary purpose is to advise the Director of the National Cancer Institute (NCI) on matters concerning the Institute's program to identify and to evaluate environmental carcinogens to which humans may be exposed. Besides addressing specific activities of the program, the Clearinghouse advises on the program's structure, direction, priorities, resources, and other areas deemed appropriate. The Clearinghouse is also responsive to requests for advice from Congress, the President's Cancer Panel, the National Cancer Advisory Board, and Federal agencies.

The Clearinghouse was established to meet a number of immediate and long-term program needs. One was the need to have greater public input into the program, especially by representatives from the major communities directly concerned with environmental carcinogenesis. Another was the need to have broader-based scientific participation in both specific and general issues involving carcinogen bioassay. Besides attempting to establish a coalition of individuals from the major communities concerned with environmental causes of cancer, the Clearinghouse membership also had to be balanced in terms of the expertise required to advise the program on technical and scientific issues. Thus, members were drawn from academia, industry, organized labor, public interest groups, and State Health officials and were selected based on their expertise and experience in carcinogenesis or related fields. Collectively they provide expertise in laboratory animal sciences, chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of twelve government agencies also participate in Clearinghouse activities as ad hoc members.

The 30 members of the Clearinghouse operate through four subgroups. One is responsible for advising on environmental chemicals or classes of chemicals that require carcinogenicity testing. Another considers appropriate experimental designs for the conduct of routine tests and advises on special protocols for studies to improve carcinogen bioassay methodology. A third subgroup evaluates the carcinogenicity of chemicals tested in the program and assesses human risk (not chemicals tested in the program and assesses human risk (not risk vs. benefit) for those adjudged to be carcinogens. The fourth subgroup is comprised of members of the other three and is responsible for providing overall direction and coordination, as well advising on matters not within the mandate of one of the other subgroups.

The first Clearinghouse meeting, a plenary session, was held in November, 1976. As of the end of the current fiscal year, the Chemical Selection and Data Evaluation/Risk Assessment Subgroups have each met five times and the Experimental Design Subgroup four times. During the meetings,

specific items within each subgroup's mandate were identified and defined and priorities for their consideration set. Certain areas in which advice is rendered, e.g., chemical selection, require the continuous review of environmental chemicals so as to ensure that those chosen for test are the ones of most concern to public health. Other areas require advice as to how the state-of-the-art can be further advanced, e.g., experimental design and risk assessment.

Present members of the Clearinghouse are: Dr. Arnold L. Brown (chairman), Mayo Clinic; Mr. Louis S. Beliczky, United Rubber Workers International, AFL-CIO, CLC; Dr. David B. Clayson, Eppley Institute for Research in Cancer; Prof. Jerome Cornfield, George Washington University; Mr. Lawrence Garfinkel, American Cancer Society; Dr. E. Cuyler Hammond, American Cancer Society; Dr. Robert W. Harkins, Grocery Manufacturers of America, Inc.; Dr. Joseph H. Highland, Environmental Defense Fund; Dr. Marvin Kuschner, State University of N.Y. at Stony Brook; Dr. William Lijinsky, Frederick Cancer Research Center; Dr. Peter N. Magee, Fels Research Institute; Dr. Elizabeth C. Miller, University of Wisconsin Medical School; Dr. Norton Nelson, New York University Medical Center; Dr. Paul Nettesheim, Oak Ridge National Laboratories; Dr. Henry C. Pitot, McArdle Laboratory for Cancer Research; Dr. Verne A. Ray, Pfizer Medical Research Laboratory; Dr. George Roush, Monsanto Company; Dr. Verald K. Rowe, Dow Chemical U.S.A.; Mr. Sheldon W. Samuels, Industrial Union Dept., AFL-CIO; Dr. Michael B. Shimkin, University of California at San Diego; Dr. Louise Strong, University of Texas Health Sciences Center; Dr. Paul O. P. Ts'o, Johns Hopkins University; Dr. I. Bernard Weinstein, Columbia University; Dr. John H. Weisburger, American Health Foundation; Dr. Gerald Wogan, Massachusetts Institute of Technology; Dr. Kenneth Wilcox, Michigan State Health Department; and Dr. Sidney Wolfe, Health Research Group. Dr. James M. Sontag, DCCP, serves as Executive Secretary of the Clearinghouse parent body and Data Evaluation/Risk Assessment and Executive Subgroups and Dr. J. Dan Recer, DCCP, as Executive Secretary of the Chemical Selection and Experimental Design Subgroups.

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14. Kraybill, H. F. and Mehlman, M. A. (Eds.), Advances in Modern Toxicology: Environmental Cancer. Hemisphere Publ. Co., Washington, D.C., 1977.

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CONTRACT NARRATIVE
OFFICE OF THE DIRECTOR
DCCP, NCI

NATIONAL INSTITUTE OF OCCUPATIONAL SAFETY AND HEALTH (YO1-CP-60605)

Title: Conduct of Research on Occupational Carcinogenesis

Contractor's Project Director: Dr. John Finklea

Project Officer (NCI): Dr. James A. Peters

Objectives: To develop a cooperative research program with the National Cancer Institute to study the effects of carcinogens in the work place. This project consists of 25 projects (1) Mortality Study of Miners Exposed to Amphibole Mineral; (2) Mortality of Pesticide Formulators; (3) Development of Sampling and Analytical Methods in Carcinogens; (4) Inhalation Study of Dibromoethane in Rats in Combination with Disulfiram; (5) Occupational Cancer Surveillance; (6) Kepone Registry; (7) Industrial Hygiene and Morbidity Study of Talc and Other Fibrous Minerals; (8) Mortality, Medical and Industrial Hygiene Study of Styrene-Butadiene Rubber Workers; (10) Mortality and Industrial Hygiene Study of PCB: (11) Industrial Hygiene Study of Azo Dye Workers; (12) Mortality and Industrial Hygiene Study of Trichloroethylene (TCE); (13) Mortality and Industrial Hygiene Study of Perchloroethylene (PCE); (14) Mortality, Medical and Industrial Hygiene Study of Chlorinated Hydrocarbons; (15) Study of Beryllium Workers; (16) Mortality and Industrial Hygiene Study of Nitrosamines/Cutting Oils; (17) Mortality and Reproductive Effects of Chloroprene; (18) Mortality, Medical and Industrial Hygiene Study of the Painting Trades; (19) Mortality and Industrial Hygiene Study of Printing Trades; (20) Mortality and Industrial Hygiene Study of Phosphate Workers; (21) Occupational Carcinogenesis Reduction by Behavorial Methods; (22) Inhalation Study of Short Asbestos Fibers; (23) Perform Subtrachial Testing of Copper and Lead Smelter Dusts (24) Determine the Effects of Combined Exposures to Smelter Dusts and SO_2; (25) Evaluation of Personal Protective Equipment.

Major Findings: No major findings have been reported as yet by NIOSH.

Significance to Biomedical Research and the Program of the Institute: The first individuals exposed to new chemical substances in significant quantity are usually workers employed in the manufacturing of the substance and its formulation into products. Therefore, the toxic or carcinogenic manifestations of a substance are often seen first in workers. Therefore, it is important to monitor the health status of workers exposed to substances expected to be carcinogens. These studies are of three types: industrial hygiene, morbidity and mortality studies of workers employed in industries which use suspect carcinogens, attempts at reduction of the effects of carcinogens by devising protective

equipment and procedures, and bioassays of mixtures suspected of special carcinogenic hazard in the work place.

Proposed Course of Project: This interagency agreement is an attempt to develop a collaborative program in occupational carcinogenesis, between NIOSH and NCI. Its further course will follow the most fruitful lines of collaboration that are developed during its initial year.

Data Contract Initiated: September 23, 1976.

Current Annual Level: $3,977,800.

CONTRACT NARRATIVE
OFFICE OF THE DIRECTOR
DCCP, NCI

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION (YO1-CP-60208)

Title: Processing Solar Ultraviolet Data

Contractor's Project Director: Dr. Lester Machta

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To process solar ultraviolet (UV) radiation data from Robertson-Berger sunburning UV (SUV) meters for up to 20 stations; and to provide the National Cancer Institute with computer tape and printouts of the processed and summarized data.

Major Findings: No findings per se by the Contractor are anticipated. Three magnetic tapes and machine printouts comprising data for 18 stations for 1975 and part of 1976 have been delivered to NCI (ADPMS, Biometry Branch) thus far.

Significance to Biomedical Research and the Program of the Institute: The data will be used to make further comparisons with skin cancer survey data similar to the study reported on in DHEW No. (NIH) 76-1029 "Measurements of Ultraviolet Radiation in the United States and Comparisons with Skin Cancer Data," by Joseph Scotto, Thomas R. Fears, and Gio B. Gori of the Division of Cancer Cause and Prevention, NIH.

Proposed Course of Project: Continuation of data processing, including the remainder of the 1976 data and as much of the 1977 data as is available before the end of the contract period, December 16, 1977.

Date Contract Initiated: February 17, 1976.

Current Annual Level: $5,000.

Annual Report
Smoking and Health Program
Division of Cancer Cause and Prevention
National Cancer Institute
July 1, 1976 through September 30, 1977

Dr. Gio B. Gori, Director

Introduction

Since 1968, the National Cancer Institute's Smoking and Health Program has been a leading force in this country in efforts to mitigate the deleterious effects of smoking on health. This important work has advanced significantly because of the quality of guidance provided by the advisory bodies that have directed the Program (the Tobacco Working Group and the Smoking and Health Review Board) and because of the contributions of scores of dedicated scientists and administrators. The close collaboration of the National Heart, Lung, and Blood Institute and the U.S. Department of Agriculture has broadened the scope of research and contributed substantially to balanced findings.

With the cooperation of both the cigarette industry and a well-informed public, full flavored, low-hazard cigarettes can be successfully marketed in the next few years. The health risk from moderate consumption of these cigarettes should be significantly below that associated with today's sales-weighted average cigarette. This optimism is supported by epidemiologic evidence, by the success of researchers in determining the characteristics of experimental less hazardous cigarettes, and by the advances in flavor and acceptability technology. In fact, forerunners of these low-hazard cigarettes have already been introduced on the American market, and, according to sales figures, some of them have been quite acceptable to the public.

Because of the multiple interactions of smoking and health and because less hazardous cigarettes are now practical and no longer only research models, this field of study offers a major opportunity in preventive medicine, one that should dramatically affect the epidemiology and public health statistics of the coming decades.

This report summarizes the progress made toward achieving the Smoking and Health Program objective of reducing the morbidity and mortality of smoking-related diseases and describes the proposed research needed to further this aim.

Background

The overall objective of the Smoking and Health Program is to reduce the morbidity and mortality of smoking-related disease. This requires dual public action to:

- Promote public awareness of smoking hazards.

- Reduce the risk to those who continue to smoke.

The first of these actions has been undertaken through public education campaigns sponsored by the National Clearinghouse for Smoking and Health (now part of the Center for Disease Control, Department of Health, Education and Welfare), the American Cancer Society, the American Heart Association, and other private organizations. The efforts of these groups are continuing and include programs for special segments of the population, e.g., pregnant women and school-age children.

The National Cancer Institute's Smoking and Health Program has undertaken the second type of action. In collaboration with the National Heart, Lung, and Blood Institute and the U.S. Department of Agriculture, the National Cancer Institute has addressed itself specifically to the problems of 1) identifying the individuals most likely to develop smoking-related diseases, 2) developing means of reducing the risks of such individuals, and 3) designing less hazardous cigarettes.

The importance of the Program objective is illustrated in Figure 1, which shows the increases in mortality due to cancers of the trachea, bronchus, and lung and which projects even further increases. These types of cancer are only a few of the diseases associated with tobacco consumption; other tobacco-related diseases add substantially to the values shown in Figure 1. To reduce the morbidity and mortality of all diseases, two approaches are used (Figure 2).

The first approach involves the modification of smoking products for the purpose of producing less hazardous cigarettes. This entails the following sequence of events:

- Tobacco blends used in experimental cigarettes are analyzed to determine their chemical constituents.

- Cigarette smoke condensate, whole smoke, and particular chemical fractions or constituents are bioassayed to obtain evidence of tumorigenicity and other biological activity. Several animal models are used to test biological parameters.

- Flavor additives are investigated to define appropriate acceptability levels within the experimental cigarette series.

- Bioassay procedures are scheduled to assess whether toxicity can result from reintroducing flavor components to a minimum cigarette configuration.

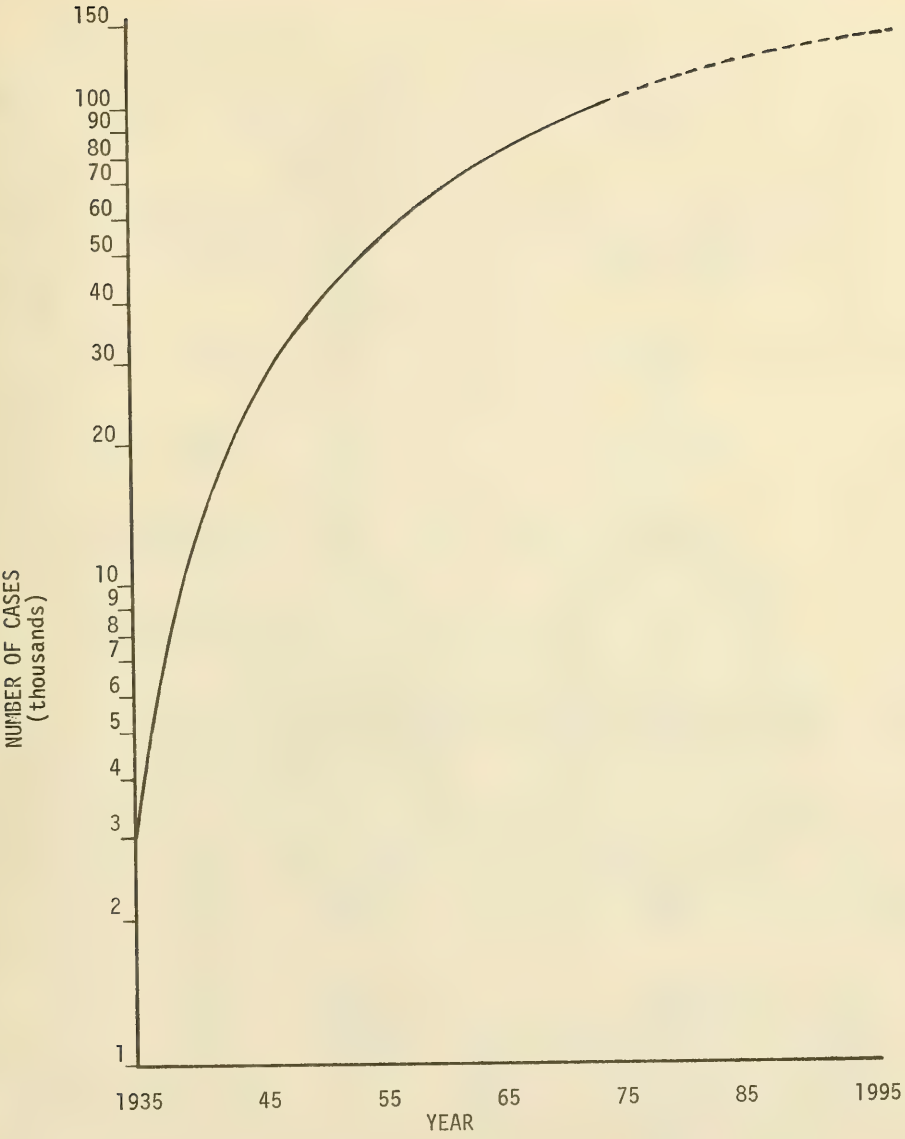


Figure 1. Trend in incidence of tracheal, bronchial, and lung cancer. Solid line is based on reported data; broken line denotes extrapolated data.

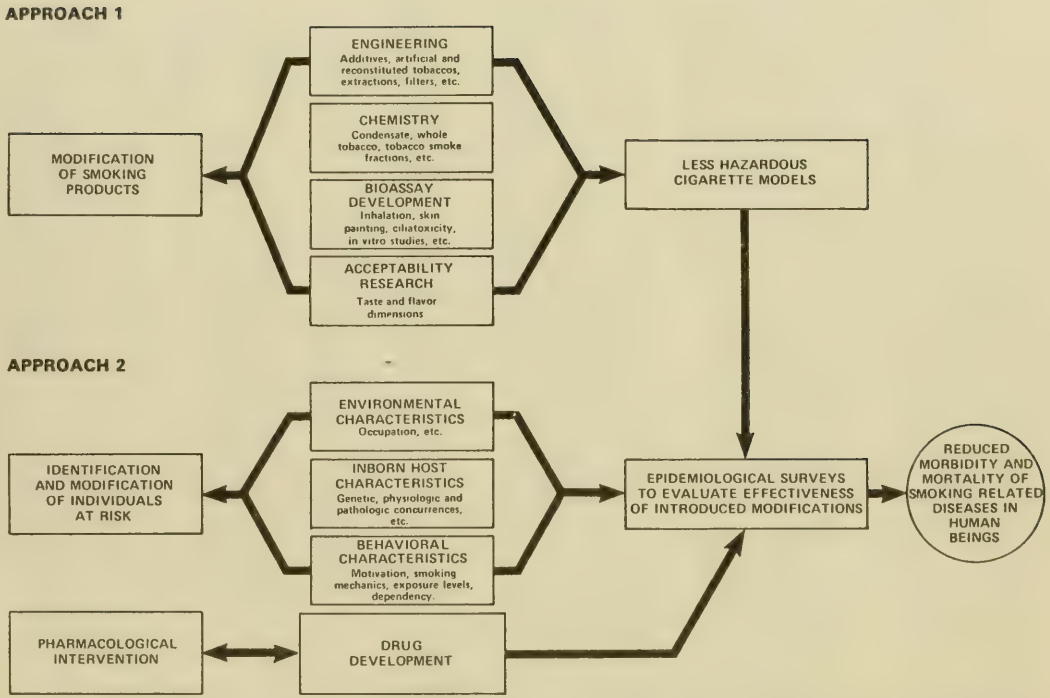

APPROACH 1
ENGINEERING
Additives, artificial and reconstituted tobaccos, extractions, filters, etc.
CHEMISTRY
Condensate, whole tobacco, tobacco smoke fractions, etc.
BIOASSAY DEVELOPMENT
Inhalation, skin painting, ciliatoxicity, in vitro studies, etc.
ACCEPTABILITY RESEARCH
Taste and flavor dimensions
MODIFICATION OF SMOKING PRODUCTS
LESS HAZARDOUS CIGARETTE MODELS
APPROACH 2
ENVIRONMENTAL CHARACTERISTICS
Occupation, etc.
INBORN HOST CHARACTERISTICS
Genetic, physiologic and pathologic concurrences, etc.
BEHAVIORAL CHARACTERISTICS
Motivation, smoking mechanics, exposure levels, dependency.
IDENTIFICATION AND MODIFICATION OF INDIVIDUALS AT RISK
EPIDEMIOLOGICAL SURVEYS TO EVALUATE EFFECTIVENESS OF INTRODUCED MODIFICATIONS
REDUCED MORBIDITY AND MORTALITY OF SMOKING RELATED DISEASES IN HUMAN BEINGS
PHARMACOLOGICAL INTERVENTION
DRUG DEVELOPMENT

Figure 2. Research plan to decrease the risk of smoking-related cancers and diseases.

- Results from chemical and biological analyses are correlated, to define further appropriate modifications of tobacco products. Such modifications should eventually result in a tobacco product and/or experimental blend that is both less hazardous and more acceptable to smokers.

The second approach involves identification of and provision for assistance to those individuals at an increased risk of tobacco-related diseases. Investigations are conducted to determine the environmental, biological, and psychological characteristics of such individuals. Epidemiological surveys are analyzed to determine those factors indicative of susceptibility to tobacco-related diseases.

From the information obtained, priorities are established for specific studies. The main lines of research are directed toward the following diseases.

- Lung Cancer -- to reduce the activity of tobacco smoke carcinogens in particulate matter and gas phase of smoke.

- Respiratory Disease -- to reduce major irritants, with particular attention being given to total particulate matter, nitrogen oxides, hydrogen cyanide, and acrolein.

- Cardiovascular Disease -- to reduce the levels of carbon monoxide and nicotine.

The influence of genetic and behavioral characteristics on susceptibility to the above-mentioned diseases is also investigated.

Interdisciplinary Cooperation

The health and technological problems connected with tobacco and tobacco smoke are complex and cross a variety of disciplines and interests. For instance, it is to the advantage of tobacco farmers and a function of the U.S. Department of Agriculture to foster research on agronomic varieties of tobacco that may result in less harmful smoking products, as evaluated by agencies with the necessary competence, such as the National Cancer Institute and the National Heart, Lung, and Blood Institute.

The improvement in some cigarette characteristics may possibly result in the worsening of others; close collaboration of experts in carcinogenesis, cardiovascular and respiratory diseases, and tobacco technology is mandatory to ensure that cigarettes become less hazardous for as many as possible of their potential effects in man. The National Cancer Institute coordinates its efforts with those of the National Heart, Lung, and Blood Institute and other public health agencies to develop smoking withdrawal techniques for the high-risk smoker and to identify individuals at risk due to inborn characteristics and environmental factors.

Developing specifications for less hazardous cigarette models and for the attendant biological and chemical analyses requires the assistance of individuals qualified in a wide spectrum of disciplines. Thus, the Smoking and Health Program supplements the expertise represented on the Smoking and Health Review Board and on the Tobacco Working Group with consultants from government, academia, and industry. Depending on the specialty required, these individuals are invited to participate in special meetings that address particular, rapidly changing, or high priority issues in smoking and health. The Smoking and Health Review Board members, the Tobacco Working Group members, and the Smoking and Health Program consultants are listed below. The major workshops and meetings supported by the Smoking and Health Program during the past 15 months are given in Table 1.

Smoking and Health Review Board

Dr. Joseph F. Fraumeni, Jr.
National Cancer Institute

Dr. Harry W. Hays
U.S. Department of Agriculture

Dr. Gardner C. McMillan
National Heart, Lung, and Blood Institute

Dr. Mearl F. Stanton
National Cancer Institute

Dr. T. C. Tso
U.S. Department of Agriculture

Tobacco Working Group

1968 - 70	Carl G. Baker, Department of Health, Education and Welfare
1972 - 75	William W. Bates, Liggett & Myers Tobacco Company
1976 -	Sam P. Battista, Arthur D. Little, Inc.
1968 - 76	Fred G. Bock, Roswell Park Memorial Institute
1968 - 73	Roswell K. Boutwell, University of Wisconsin
1968 - 70	Hans L. Falk, National Institute of Environmental Health Sciences
1973 -	Alfred Fishman, Hospital of the University of Pennsylvania
1974 - 75	Jean D. Gibbons, University of Alabama
1969 -	Gio B. Gori, National Cancer Institute
1972 - 74 1976 -	Michael R. Guerin, Oak Ridge National Laboratory
1974 -	Ian Higgins, University of Michigan
1973 - 75	Dietrich Hoffmann, American Health Foundation
1972 - 74 1976	I. W. Hughes, Brown & Williamson Tobacco Corporation
1968 - 76	Charles J. Kensler, Arthur D. Little, Inc.
1968 - 69	Paul Kotin, John Mansville, Inc.
1973 -	Henry C. McGill, Jr., University of Texas
1969 -	Gardner C. McMillan, National Heart, Lung, and Blood Institute
1968 - 70	Ian A. Mitchell, Department of Health, Education and Welfare
1972 -	Thomas B. Owen, National Cancer Institute
1976 -	Alan Rodgman, R. J. Reynolds Tobacco Company
1968 - 74	Umberto Saffiotti, National Cancer Institute
1968 - 74	Marvin A. Schneiderman, National Cancer Institute
1976 -	Robert B. Seligman, Philip Morris, U.S.A.
1972 - 74	Irving J. Selikoff, Mt. Sinai School of Medicine
1968 - 76	Murray Senkus, R. J. Reynolds Tobacco Company
1974 - 75	Philippe Shubik, Eppley Institute for Research in Cancer
1968 -	A. W. Spears, Lorillard Research Center
1968 - 69	Jesse L. Steinfeld, Medical College of Virginia
1968 -	T. C. Tso, U.S. Department of Agriculture
1968 - 74	Benjamin L. Van Duuren, New York University Medical Center
1968 - 76	Helmut Wakeham, Philip Morris, U.S.A.
1968 - 73	Ernst L. Wynder, American Health Foundation

Smoking and Health Program Consultants

Dr. Fawky Abdallah
Imperial Tobacco Limited

Dr. Alan Armitage
Council for Tobacco Research

Dr. Mark Armstrong
University of Iowa

Dr. Oscar Auerbach
Veterans Administration Hospital, N.J.

Dr. John Bailar
National Cancer Institute

Dr. James Bain
Emory University

Dr. Carl G. Baker
Hazleton Laboratories America, Inc.

Dr. David Bates
University of British Columbia

Dr. William W. Bates
Liggett & Myers Tobacco Company

Dr. Mario Battigelli
University of North Carolina

Dr. Sam P. Battista
Arthur D. Little, Inc.

Dr. John F. Benner
University of Kentucky

Dr. John Berg
University of Iowa

Dr. Edwin Boatman
University of Washington

Dr. Fred G. Bock
Roswell Park Memorial Institute

Dr. Roswell K. Boutwell
University of Wisconsin

Dr. Edward Bresnick
Medical College of Georgia

Dr. Kenneth Brinkhous
University of North Carolina

Dr. Stuart Brooks
Department of Environmental Health, Cincinnati

Dr. John Caton
Oak Ridge National Laboratory

Dr. Ercoli Cavalieri
University of Nebraska

Dr. James F. Chaplin
Tobacco Research Laboratory

Dr. O. T. Chortyk
Richard B. Russell Research Center

Dr. Thomas B. Clarkson
Bowman-Gray School of Medicine

Dr. Richard Corlett
University of Washington

Dr. Morton J. Cowan
National Heart, Lung, and Blood Institute

Dr. John Craighead
University of Vermont

Dr. Signey Cutler
Georgetown University School of Medicine

Dr. Gerald Deneau
University of California School of Medicine

Dr. Louis Diamond
University of Kentucky

Dr. Edward F. Domino
University of Michigan

Dr. Grace M. Donnelly
University of Kentucky

Dr. John Edward Dunn
California Department of Public Health

Dr. John T. Edsall
Harvard University

Dr. Gerald F. Egan
Exxon Corporation

Dr. Ronald Estabrook
University of Texas

Dr. Hans L. Falk
National Institute of Environmental Health Sciences

Dr. Alfred Fishman
Hospital of the University of Pennsylvania

Dr. Robert Forster
Hospital of the University of Pennsylvania

Dr. F. John Francis
University of Massachusetts

Dr. Joseph F. Fraumeni, Jr.
National Cancer Institute

Dr. Perry J. Gehring
Dow Chemical Company

Dr. Jean D. Gibbons
University of Alabama

Dr. Michael R. Guerin
Oak Ridge National Laboratory

Mr. Howard M. Halter
AMF Incorporated

Dr. E. Cuyler Hammond
American Cancer Society

Dr. Naomi Harley
New York University Medical Center

Dr. John Hayes
Mallory Institute

Dr. Harry W. Hays
U.S. Department of Agriculture

Dr. Allen Heim
National Cancer Institute

Dr. John Heller
National Cancer Institute

Dr. Ian Higgins
University of Michigan

Dr. Peter Hill
American Health Foundation

Dr. Marcus E. Hobbs
Duke University

Dr. Dietrich Hoffmann
American Health Foundation

Dr. Evan C. Horning
Baylor College of Medicine

Dr. I. W. Hughes
Brown & Williamson Tobacco Corporation

Dr. Robert E. Hyatt
Mayo Clinic

Dr. Ronald H. Ingram
Peter Bent Brigham Hospital

Dr. Thomas N. James
University of Alabama Medical Center

Dr. Murray E. Jarvik
VA Brentwood Hospital

Dr. Morley Kare
Monell Chemical Senses Center

Dr. Charles H. Keith
Celanese Fibers Company

Dr. Kenneth Keller
North Carolina State University

Dr. Ann Kennedy
Harvard School of Public Health

Dr. Charles J. Kensler
Arthur D. Little, Inc.

Mr. Robert L. Kersey
Liggett & Myers Tobacco Company

Dr. Irving Kessler
Johns Hopkins University

Dr. Jerome Kleinerman
St. Luke's Hospital

Dr. Francis Klocke
Buffalo General Hospital

Dr. George B. Koelle
University of Pennsylvania School of Medicine

Dr. Bruce Kottke
Mayo Clinic

Dr. B. K. Krotoszynski
IIT Research Institute

Dr. H. Kubota
Oak Ridge National Laboratory

Dr. Marvin Kuschner
State University of New York at Stony Brook

Dr. Louis Lasagna
University of Rochester

Mr. Thomas Lee
National Bureau of Standards

Dr. Claude J. Lenfant
National Heart, Lung, and Blood Institute

Dr. William Lijinsky
Oak Ridge National Laboratory

Mr. Clifford Lilly
Philip Morris, U.S.A.

Dr. John P. Long
University of Iowa

Dr. Harold N. MacFarland
Gulf Oil Corporation

Dr. Robert Mahley
National Heart, Lung, and Blood Institute

Dr. Owen Maller
Monell Chemical Senses Center

Dr. Charles Mattina
Peter J. Schweitzer Division

Dr. Bruce McCullough
Southwest Foundation for Research and Education

Dr. Henry C. McGill, Jr.
University of Texas

Dr. C. A. McMahan
University of Texas

Dr. Gardner C. McMillan
National Heart, Lung, and Blood Institute

Dr. Harold Menkes
Johns Hopkins University School of Medicine

Smoking and Health Program Consultants (Cont'd)

Dr. Sanford A. Miller
Massachusetts Institute of Technology

Dr. Ralph Mitchell
Battelle Memorial Institute

Dr. E. L. Moore
U.S. Department of Agriculture

Dr. Gerald P. Morie
Tennessee Eastman Company

Dr. Kenneth Moser
University Hospital of San Diego County

Dr. Jay Moskowitz
National Heart, Lung, and Blood Institute

Dr. Robert Murphy
University of Colorado Medical School

Dr. Paul Nettesheim
Oak Ridge National Laboratory

Dr. Mark Nickerson
McGill University

Dr. Thomas Osdene
Philip Morris, U.S.A.

Dr. Philip Pratt
Duke University Medical Center

Dr. Osgood D. Priddle
Bausch and Lomb

Dr. James R. Prine
Los Alamos Scientific Laboratory

Dr. Roger L. Priore
Roswell Park Memorial Institute

Dr. Donald F. Proctor
Johns Hopkins School of Hygiene

Dr. Russell Prough
University of Texas

Dr. K. Pump
University of British Columbia

Dr. Gunter Rathkamp
Waters Mestenick G.m.b.H.

Dr. William K. Riker
University of Oregon Medical School

Dr. W. E. Rinehart
Bio/Dynamic, Inc.

Dr. Abel L. Robertson, Jr.
Case Western Reserve University

Dr. John Rockett
National Bureau of Standards

Dr. Alan Rodgman
R. J. Reynolds Tobacco Company

Dr. Geno Saccomanno
St. Mary's and V.A. Hospitals

Dr. Umberto Saffiotti
National Cancer Institute

Dr. Irwin Schmeltz
American Health Foundation

Dr. Marvin A. Schneiderman
National Cancer Institute

Dr. Frederick J. Schultz
Lorillard Research Center

Dr. Robert B. Seligman
Philip Morris, U.S.A.

Dr. Irvin J. Selikoff
Mt. Sinai School of Medicine of the City University of New York

Dr. William Selke
Kimberly-Clark Corporation

Dr. Murray Senkus
R. J. Reynolds Tobacco Company

Dr. Michael B. Shimkin
University of California, San Diego

Dr. A. W. Spears
Lorillard Research Center

Dr. Mearl F. Stanton
National Cancer Institute

Dr. Jesse L. Steinfeld
Mayo Clinic

Dr. G. W. Stokes
University of Kentucky

Dr. James R. Stokely
Oak Ridge National Laboratory

Dr. Jack T. Strong
Louisiana State University
Medical School

Dr. Martin Summerfield
Princeton University

Dr. Philip S. Thayer
Arthur D. Little, Inc.

Dr. Wilbur Thomas
Albany Medical College

Dr. Bela Toth
University of Nebraska

Dr. Richard E. Tracy
Louisiana State University

Dr. T. C. Tso
U.S. Department of Agriculture

Dr. Walter S. Tyler
University of California

Dr. Benjamin L. Van Duuren
New York University Medical Center

Dr. Helmut Wakeham
Philip Morris, U.S.A.

Dr. Lee Wattenberg
University of Minnesota

Dr. Elizabeth Weisburger
National Cancer Institute

Dr. Otto Wong
Georgetown University School of
Medicine

Dr. John Wyatt
University of Kentucky

Dr. Ernst L. Wynder
American Health Foundation

Dr. Stanley Yamashiro
University of Southern California

Dr. Donovan Yeates
New York University

Dr. Raymond Yesner
Veterans Administration Hospital,
Conn.

Table 1

Major Workshops and Meetings Supported
by the Smoking and Health Program

July 1976 - September 1977

Workshop on Polonium-210
Workshop on Series V Variants
Tobacco Working Group Meeting (September 8, 1976)
Tobacco Working Group Meeting (March 16, 1977)
Tobacco Working Group, Chemistry Subcommittee Meeting
Meeting to Discuss Design and Effectiveness of Smoking Machines
Phase Separation and Chemical Analysis Meeting on Cytrel
Symposium on Bioassay Models for Inhalation Toxicology
Workshop on Bioactivity of Ozone and Nitrogen Oxides
Plus 28 meetings with NCI, legal and special purpose meetings

Program Management and Resource Allocation

Dr. Gio B. Gori and Dr. Thomas B. Owen of the National Cancer Institute are the Director and Assistant Director, respectively, of the Smoking and Health Program. They supervise the overall program, provide scientific guidance, and monitor projects.

Program management and support are provided by the Prime Contractor, Enviro Control, Inc., whose responsibilities include overall program coordination, activities in support of the program office, and direct management of all subcontracts assigned to it. The National Cancer Institute is directly responsible for interagency agreements and retains control of all major scientific and policy decisions. The organizational scheme is illustrated in Figure 3.

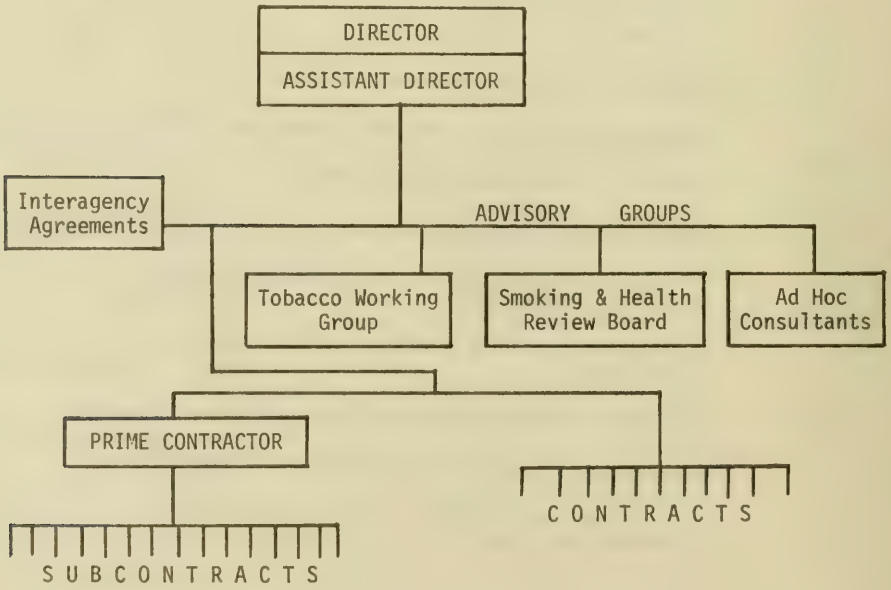


Figure 3. Organizational chart for the National Cancer Institute's Smoking and Health Program.

The allocation of resources for the Smoking and Health Program is delineated in Table 2 and Figure 4. Table 2 lists contracts by type (interagency, National Cancer Institute contractor, and prime contractor and subcontractor), whereas Figure 4 illustrates the distribution of effort by research area. Descriptions of individual projects are given in a later section of this report.

Current Activities and Accomplishments

The activities discussed in this section represent efforts either to develop a less hazardous cigarette or to identify those factors that increase individual risk for tobacco-related diseases.

During the past 15 months, funds were allocated for 32 projects involving various aspects of tobacco-related research. Of these, 14 projects (including 6 interagency agreements) are under the direct management of the National Cancer Institute. The remaining 18 projects, contracted with nongovernmental institutions, are under the management of Enviro Control, Inc.

Tables 3a and 3b list these 32 projects in the context of program objectives. Table 4 describes those results that have had a significant impact on the overall program. In examining these tables, one should keep in mind that individual projects may cover more than one of the categories used. However, to facilitate presentation of the status of the Smoking and Health Program, projects and observations have been assigned to only one category within the tables. Detailed descriptions in the project narratives indicate the broader work scope of each study.

The balance of this section of the report discusses program status as described in Table 4, within the categories shown in Table 3a.

Chemical and Biochemical Analyses. The Smoking and Health Program procures experimental tobacco blends for testing in candidate less hazardous cigarettes. The projects discussed in the following paragraphs (see Table 3a) represent a collaborative effort to identify the major chemical components of the tobacco in and of the smoke from these blends, as well as the biochemical fate and metabolic products of the major smoke constituents.

Experimental modification of cigarettes has taken place since 1950. Certain consistent results led to reasoned predictions of the effects of certain modifications on tumorigenicity. In 1970, under the direction of the Tobacco Working Group, researchers defined the first of several series of experimental cigarette blends, using these early findings as a basis.

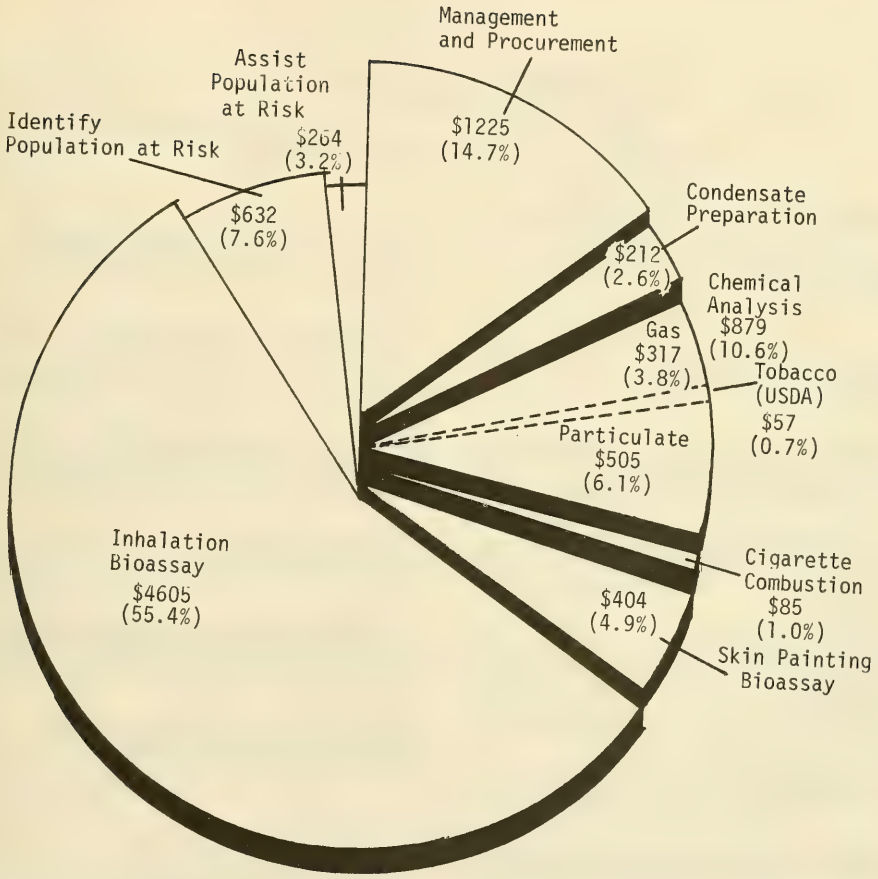
Series I was designed to test variables of cigarette engineering (e.g., variations in paper porosity and widths of tobacco cuts). Other variables tested were nitrate concentrations, fractions of blends (such as leaves or stems only), and reconstituted tobacco sheets. Included in this and each of the following series were two standard blends for series cross-comparisons. These were the 1R1 developed by the University of Kentucky and a standard experimental blend (SEB) designed to represent the average tobacco composition in 1970.

Table 2

Current Resource Allocation
Smoking and Health Program
October 1, 1976 - September 30, 1977
(Ongoing and New Projects)[a]

NCI Administrative Division	Contractor/Division	Cost	Program Percentage
Interagency Agreements			
Chemistry, Tobacco	USDA	57	
Chemistry, Gas	ORNL	188	
Chemistry, Particulate		376	
Inhalation Bioassay/Dogs	V.A.(N.J.)	359	
Development Inhalation Bioassay	ORNL	184	
Pulmonary Screening/Clinical	V.A. (Miami)	20	
	5	1,184	14.3
NCI Contractors			
Inhalation Bioassay/Dogs	BNW	707	
Lung Pellet Bioassay/Rats	BNW	25	
	2	732	8.8
Prime Contractor & Subcontractors			
Management & Procurement	ECI	1,225	
Condensate Preparation	Meloy	212	
Chemistry, Gas	AHF	129	
Chemistry, Particulate	AHF	129	
Bioassay, Skin Painting	AHF	26	
Epidemiology, National	AHF	364	
Epidemiology, Multinational	Europe (6)	250	
Epidemiology, Cuban	IARC	18	
Cigarette Combustion	Princeton U.	85	
Bioassay, Skin Painting	HAZ	345	
Bioassay, Sebaceous Gland	Ros. Pk. Inst.	8	
Bioassay, Cytotoxicity	ADL	126	
Develop. Inhalation Model/Baboons	SFRE	103	
Bioassay, Inhalation/Rats	BNW	660	
Bioassay, Inhalation/Dogs	ECI	1,614	
Bioassay, Inhalation/Dogs	HAZ	604	
Bioassay, Inhalation, Atherosclerosis/Dogs	HAZ	75	
Bioassay, Inhalation, Atherosclerosis/Pigeons	Harvard U.	88	
Pulmonary Screening/Clinical	J.H.U.	150	
Pharmacology, Clinical Trials	AHF	50	
Develop. Dosimetry Equip. & Tech.	ADL	85	
Cigarette Acceptability/Smokers	ADL	44	
	25	6,390	76.9
TOTAL PROGRAM	32	8,306	100.0

[a]All cost figures annualized. Values expressed as estimated funds (x 1000).



All dollar Figures x 1000
Total Funding $8306
All Figures Annualized

Figure 4. Current resource allocation of the Smoking and Health Program for Fiscal Year 1977 (Oct. 1, 1976-Sept. 30, 1977).

Table 3a

Smoking and Health Program Contracts
July 1976 - September 1977

DEVELOPMENT OF A LESS HAZARDOUS CIGARETTE

Chemical and Biochemical Analyses

American Health Foundation New York, New York SHP-74-106 Part II	Evaluation of Carcinogenic Agents in Cigarette Smoke: Laboratory Studies
Arthur D. Little, Inc. Cambridge, Massachusetts SHP-75-116	Development of a Flavor System for Acceptability to Smokers of Candidate Less Hazardous Cigarettes
ERDA-Oak Ridge National Laboratory Oak Ridge, Tennessee YO1-CP6-0206	Collection, Separation, and Elucidation of the Components of Cigarette Smoke and Smoke Condensate
Meloy Laboratories, Inc. Falls Church, Virginia SHP-75-118	Preparation and Analysis of Cigarette Smoke Condensate Samples
Princeton University Princeton, New Jersey SHP-75-108	A Computer-Simulated Cigarette Model for Use in the Development of Less Hazardous Cigarettes
U.S. Department of Agriculture Beltsville, Maryland YO1-CP6-0209	Modification of Tobacco Through Production and Curing Process

Bioassay for Tumorigenic Activity

Arthur D. Little, Inc. Cambridge, Massachusetts SHP-74-105-SA1	A Coordinated Program on Biological Effects of Cigarette Smoke: Short-Term *In Vitro* Bioassays, Models of Neoplasia, and Other Aspects of Carcinogenesis
Battelle Pacific Northwest Laboratories Richland, Washington NO1-CP3-3374	Research on Standardizing the Lung Pellet Implantation Technique in the Rat
Battelle Pacific Northwest Laboratories Richland, Washington NO1-CP4-3315	Inhalation Bioassay of Cigarette Smoke in Dogs: High Nicotine/ Low Nicotine

Table 3a (cont'd)

Battelle Pacific Northwest Laboratories Richland, Washington SHP-75-121	Inhalation Bioassay of Cigarette Smoke in Rats
Enviro Control Research Laboratories Temple Hills, Maryland NO1-CP5-5666-SA12	Inhalation Bioassay of Cigarette Smoke in Dogs: High- and Low-Nicotine Content
ERDA-Oak Ridge National Laboratory Oak Ridge, Tennessee YO1-CP4-0206	Cigarette Smoke Bioassay Development
Harvard University Cambridge, Massachusetts SHP-75-111	Inhalation Bioassay of Cigarette Smoke in Pigeons: Effect of Nicotine and Carbon Monoxide on Atherogenesis
Hazleton Laboratories America, Inc. Vienna, Virginia SHP-74-101	Mouse Dermal Bioassay of Cigarette Smoke Condensate
Hazleton Laboratories America, Inc. Vienna, Virginia SHP-75-112	Inhalation Bioassay of Cigarette Smoke in Dogs: Effects of Nicotine, Carbon Monoxide, and Nitrogen Oxides on Atherogenesis
Hazleton Laboratories America, Inc. Vienna, Virginia SHP-75-117	Respiratory Impairment in Beagles Exposed to Cigarette Smoke
Roswell Park Memorial Institute Buffalo, New York SHP-75-113	Bioassay of Cigarette Smoke Condensate by Sebaceous Gland Suppression Test
Southwest Foundation for Research and Education San Antonio, Texas SHP-74-102-SA1	Inhalation Testing of Cigarette Smoke in Baboons
Veterans Administration Hospital East Orange, New Jersey YO1-CP4-0205	Effects of High- and Low-Nicotine Cigarettes on Male Beagle Dogs

Table 3a (cont'd)

RISK-RELATED FACTORS

Individual Risk Analysis

American Health Foundation New York, New York SHP-74-106 Part I	Evaluation of Carcinogenic Agents in Cigarette Smoke: Epidemiological Evaluation of Carcinogenic Agents in Cigarette Smoke
Arthur D. Little, Inc. Cambridge, Massachusetts SHP-75-110	Cigarette Dosimetry in Man: Development of Equipment and Techniques
Enviro Control, Inc. Rockville, Maryland SHP-75-120	An International Epidemiologic Study of the Relationship Between Lung Cancer and Cigarette Smoking
International Agency for Research on Cancer Lyons, France SHP-76-130	Case Control Study of Lung Cancer in Cuban Women
Johns Hopkins University Baltimore, Maryland SHP-74-103	Pulmonary Screening Tests for the Effects of Cigarette Smoke on Small Airways in the Human Lung
Veterans Administration Hospital Miami, Florida YO1-CP6-0207	The Detection of Early Obstruction of Respiratory Airways

Assisting Populations at Risk

American Health Foundation New York, New York SHP-74-104B	Clinical Trials for Pharmacological Approaches to Smoking Withdrawal

MANAGEME. . SUPPORT

Enviro Control, Inc. Rockville, Maryland NO1-CP5-5666	Smoking and Health Prime Contract (includes tobacco/cigarette procurement)

Table 3b

Smoking and Health Program Contracts
Completed During the Past 15 Months

Huntingdon Research Center Brooklandville, Maryland NO1-CP4-5622	Metabolic Studies on Tobacco Smoke Constituents (HCN, NO_x, CO)
University of Maryland Baltimore, Maryland NO1-CP4-3312	Metabolic Studies on Tobacco Smoke Constituents (Nicotine)
New York University Medical Center New York, New York NO1-CP3-3241	Studies on Carcinogenic and Tumor-promoting Principles of Tobacco and Tobacco Smoke
Microbiological Associates, Inc. Bethesda, Maryland NO1-CP4-3309	Standardization of Aryl Hydrocarbon Hydroxylase
North Texas State University Denton, Texas NO1-CP5-5626	Standardization of Aryl Hydrocarbon Hydroxylase Assay as a Screening Method To Determine Smoking Hazards in Man

Table 4

Major Accomplishments of the
Smoking and Health Program

Cigarette Engineering

- Reduction of tar level is possible using reconstituted sheet processing.
- Nicotine levels and nicotine/tar ratios can be adjusted using tobacco extraction, substitution techniques, and reblending.
- Low nicotine tobacco varieties are available.
- Improved filters can be used to selectively remove certain undesirable smoke components.
- Curing and fermentation processes may induce the formation of carcinogenic constituents.
- Undesirable smoke components can be reduced or removed by any of the following: high-porosity paper, smoke dilution devices, nitrates added to the blend, and increasing the filling power of the tobacco.
- Static burn rate is an important factor in less hazardous cigarettes, but it can be manipulated only in a relatively narrow range.
- A simplified mathematical model is being developed to describe pyrolytic kinetics of tobacco and cigarettes.
- Cellulose acetate, activated charcoal, and potassium permanganate are known to be "good" filter materials.
- pH, CO, CO_2 and NH_3 correlate with inhalability levels of modified cigarettes.
- Many cigarettes already on the market have reduced CO levels.

Chemistry

- Condensate carcinogenic activity is found primarily in the polynuclear aromatic hydrocarbon fraction.
- Tumor promoters and/or cocarcinogens are found in weakly acidic and naphthalene concentrate fractions.

- Alkylfluoranthenes and alkylchrysenes contribute to tumorigenicity of tobacco smoke.
- Nicotine is most likely the major precursor of nitrosonornicotine.
- Some methylchrysenes are found to be potent complete carcinogens.
- Experimental blend cigarettes produce less CO and CO_2 per cigarette than SEB's.
- High-resolution gas chromatography has been adapted to visualize 150-200 gas-phase components.
- Techniques have been developed for recovery, isolation, and measurement of nicotine and its metabolites in minute quantities.
- Data are accumulating on the terpenoid component of condensates.
- Techniques have been developed for radioimmunoassay of nicotine and cotinine in the picogram range.
- Techniques have been developed to obtain pharmacokinetic and metabolic data on some smoke components.
- Alkyl catechols have been identified in major tumor-promoting subfractions of smoke.

Bioassay

- Tar, nicotine, and benz[a]anthracene are highly positively correlated with tumorigenicity in skin painting. Static burn rate is negatively correlated. Weak acid, phenol and benzo[a]-pyrene also correlate with skin painting results.
- Mouse skin painting data indicate substantial decrease in tumorigenicity in Series I to III cigarettes.
- HCN is found to be associated with ciliatoxicity of whole smoke.
- Correlation exists between smoke components of Series I and II and ciliatoxicity, macrophage inhibition, and cytotoxicity potency.

- Squalene, phenol, and esculin have inhibitory effects on benzo(a)pyrene carcinogenic activity but effect in condensate is not known.
- Rat, dog, and baboon inhalation bioassay techniques have been developed.
- Dog inhalation data indicate definite physiological changes resulting from chronic smoke exposure.
- Techniques for measuring pulmonary impairment in dogs have been developed.
- Cigarette smoke carcinogenic properties are being tested using sebaceous gland suppression tests.

Flavor/Acceptability

- Flavor dimensions have been identified and isolated.
- Nicotine has been identified as a major contributor to cigarette flavor acceptability.
- Flavor additives can be returned to stripped tobacco and nontobacco cigarettes to increase their acceptability.
- Based on U.S. consumption patterns, an envelope of acceptable flavor profiles has been drawn for candidate less hazardous cigarettes.
- Stripped tobaccos have been observed to produce a characteristically high level of throat irritancy during and after smoking.

Risk Reduction

- Reduction of lung cancer risk after 10 years of smoking low- instead of high-tar cigarettes is observed; also there is an observed reduced risk of cancer of larynx and oral cavity.

Table 4 (Cont'd)

- Reduction of larynx cancer risk after 10 years of smoking filter instead of nonfilter cigarettes has been observed.
- Relationship between tobacco smoking and glottic, supraglottic, and laryngeal cancers exists.
- Increased smoking consumption for filter cigarette smokers, as compared to nonfilter cigarette smokers, has been observed.
- Lung cancer risks are greater for lower educational and socioeconomic groups.
- Babington nebulizer is reliable in delivering specified drug doses in aerosol form.
- Laminar flow electrophoresis is being tested for reducing variability in AHH analysis.

Series II, initiated in 1972, applied the preliminary results of Series I bioassay and incorporated more agronomic factors. Variables of particular concern in this series included changes in amounts of tobacco per cigarette and aeration of the burning zone, use of tobacco from plants of various nicotine levels, use of various types and amounts of nitrogen fertilizer and suckering chemicals, use of tobacco with different leaf processing, and use of some artificial tobacco substitutes.

Based on the completed analyses of Series I and preliminary results from Series II, the variables to be tested in Series III and IV were defined by the Tobacco Working Group. Series III cigarettes were manufactured with the SEB and included more extensive manipulation of paper porosity and filters and changes in concentrations of sugars, humectants, and flavorings. Also included were proprietary nontobacco cigarettes.

Series IV extended the accomplishments of Series II. Through the use of extracted tobacco and the addition of SEB having specified nicotine levels, researchers were able to manipulate the nicotine level of cigarettes more closely. Other variables of concern were new processes for reconstituted tobacco sheet with and without additives and the effects of waxes. In addition, anti-irritants and proprietary nontobacco materials were included in this series.

A fifth series is now in the planning stage. It is based on the "minimum cigarette concept"--maximum simplification of tobacco smoke through new applications of established tobacco processing techniques (i.e., reconstituted sheet technology, tobacco extraction, and tobacco dilution with inert fillers). This series will be designed primarily for inhalation bioassays rather than large-scale mouse dermal bioassays.

Once produced, each series of experimental cigarettes is machine-smoked, and the condensate is collected (Meloy Laboratories, Inc.). Smoke condensates are dissolved in specified solvents, and samples are frozen for later chemical and biological assays.

These smoke condensates (as well as intact tobacco material) are chemically analyzed to make qualitative and quantitative determinations of known tobacco and tobacco smoke condensate components (American Health Foundation; Meloy Laboratories, Inc.; Oak Ridge National Laboratory; U.S. Department of Agriculture). Cigarette smoke contains many chemicals that produce tumorigenic, respiratory, and cardiovascular effects. Tumorigenic activity of smoke is related not only to concentrations of carcinogens and tumor initiators, but also to levels of cocarcinogens and tumor promoters, which enhance carcinogenic activity by as yet unknown mechanisms. Collaborative efforts of several laboratories have led to the following observations:

- Although carcinogenic activity is found primarily in the polynuclear aromatic hydrocarbon fraction of smoke condensate, tumor promoters and cocarcinogens are also found in the weakly acidic and naphthalene fractions.

- Curing and fermentation processes may induce the formation of carcinogenic components.

- The dilution filter is more effective in reducing tumorigenicity than either permanganate or cellulose acetate filters.

- Magnesium nitrate reduces cigarette smoke condensate tumorigenicity.

- Inhalability levels in modified cigarettes are correlated with pH, CO, CO_2, and NH_3 levels.

- Addition of nitrates to the blends decreases some undesirable smoke components.

- Hydrogen cyanide in whole smoke is associated with toxicity for ciliated epithelium of respiratory airways.

In addition to the above chemical analyses, a computer simulation model has been designed (Princeton University) to aid in predicting design parameters of potentially less hazardous cigarettes. The simulation model also provides a rational description of the pyrolytic kinetics, thus enabling determination of the probable smoke composition of candidate less hazardous cigarettes. Simulation models can provide a link between design parameters and the elimination of undesirable smoke components.

Metabolic pathways of particularly significant smoke constituents have been investigated, with emphasis on nicotine, HCN, carbon monoxide, and oxides of nitrogen (Huntingdon Research Center, University of Maryland). Several techniques have been devised to obtain pharmacokinetic and metabolic data on these components. Organ deposition of radioactive HCN has been determined with rats. Other metabolic products have been traced after inhalation, and the effect on biological processes has been noted.

There is little doubt that through such research a less hazardous cigarette can be designed. It is important, however, that steps be taken to ensure smoker acceptability of such a product. An effort is being made to determine the organoleptic properties associated with a series of experimental cigarettes (Arthur D. Little, Inc.), with the following results:

- Flavor dimensions have been defined.

- Acceptable flavor profiles have been compiled for candidate less hazardous cigarettes.

- Nicotine has been found to be a major contributor to cigarette flavor acceptability.

- Stripped tobacco has been shown to cause a characteristically high level of throat irritancy, both during and after the smoking process.

Bioassay for Tumorigenic Activity. The Smoking and Health Program screens the series of experimental cigarettes described above by means of a stepwise bioassay procedure. Several models are used to determine tumorigenicity levels of whole smoke and smoke constituents. Decisions as to the sequence of application of these models are made by balancing the cost of the bioassay in terms of time and dollars with the sensitivity of decision required for the assay. The decision flow is illustrated in Figure 5. The first step has been to test smoke condensate activity by use of mouse dermal bioassays (Hazleton Laboratories America, Inc.). Smoke condensates from the first four series of cigarettes have been bioassayed in this manner.

Evidence of progress in this approach is shown in Figure 6, which illustrates the results of skin painting with condensate from cigarettes in Series I through III. A trend of decreasing tumorigenicity has been found for these series when compared to SEB, indicating that cigarettes made with high-porosity paper, tobacco stems only, and reconstituted tobacco sheet are less tumorigenic than the SEB. Thus, advances are being made in designing candidate less hazardous cigarettes.

Chemical/physical parameters have been statistically analyzed against tumorigenic potency, as evidenced in skin painting experiments. Analyses of Series I, II, and III have been published in Toward Less Hazardous Cigarettes, Report Nos. 1, 2, and 3, respectively. Data indicate that benz[a]anthracene and tar are highly positively correlated with tumorigenicity. Some additional smoke components and properties are also correlated with tumorigenicity, e.g., weak acids, phenols, benzo[a]pyrene, and static burn rate. Skin painting has been initiated for the fourth series of experimental cigarettes. This group of cigarette types is expected to follow the trend of the first three series, that is, to be even less tumorigenic than those previously tested.

In addition to whole-smoke condensates, specific tobacco components and fractions are also tested by using the mouse dermal bioassay (American Health Foundation, New York University Medical Center). Specifically, agents such as catechol, pyrene, fluoranthrene, benzo[e]pyrene, benzo[g,h,i]-perylene, decane, and undecane are observed to be cocarcinogenic with benzo[a]pyrene. Data indicate that phenol, squalene, esculin, and oleic acid have inhibitory effects on the tumorigenic activity of benzo[a]pyrene.

Although mouse dermal bioassays have played an important role in the design of a less hazardous cigarette, they involve long periods of time (more than 18 months) between initial applications of condensate and verification of results. For this reason, the Smoking and Health Program has investigated the sebaceous gland suppression test as a rapid first screen bioassay. Preliminary results are promising; the test is faster, requiring only 7 days for completion, and application results in gland suppression and skin tumor development. To some extent, this correlation is independent of the concentration of benzo[a]pyrene in the various condensates. Therefore, sebaceous gland suppression provides a second criterion by which promising candidate cigarettes can be selected through short-term assays.

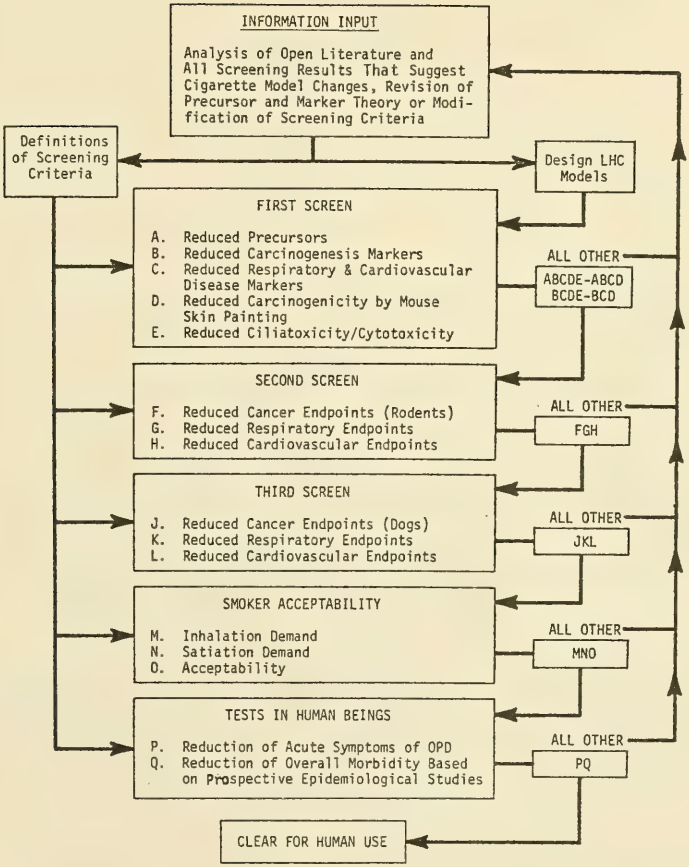


Figure 5. Development of less hazardous cigarettes: synopsis of decision flow.

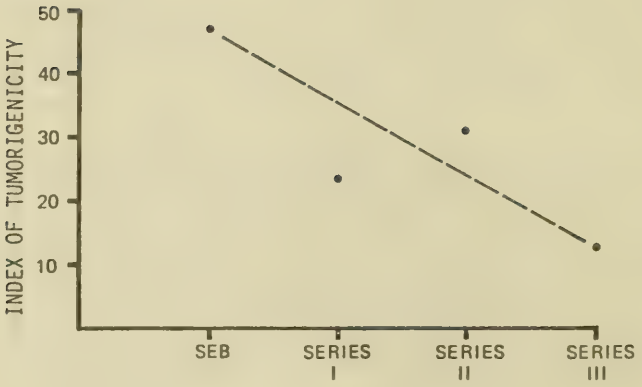


Figure 6. Toward less hazardous cigarettes: results of mouse dermal bioassay. Data were derived from dermal bioassay results for each series. SEB represents an average of the Standard Experimental Blend results for all series.

The second step in the current bioassay sequence is to test a selection of experimental cigarettes exhibiting low tumorigenic potency in mouse dermal bioassays, with whole-smoke inhalation models (Battelle Pacific Northwest Laboratories; Enviro Control Research Laboratories; Hazleton Laboratories America, Inc.; Oak Ridge National Laboratory; Veterans Administration Hospital, East Orange, New Jersey). Using automatic smoking machines, researchers have initiated the routine, chronic exposure of rats and dogs. Techniques have been developed to measure several parameters of cardiovascular and pulmonary function. Chronic exposure effects for animal models will be determined on the basis of the results of these tests and on the basis of histopathological data. Preliminary findings indicate definite functional and morphological changes related to chronic cigarette smoking. Conclusive data are not yet available.

Dog inhalation models are being used to study the apparent correlation between nicotine content and tumorigenicity of experimental cigarettes, as found in mouse dermal bioassays. Beagles are being exposed to smoke from cigarettes of high- and low-nicotine content throughout their lifespans (Veterans Administration Hospital, East Orange, New Jersey). In another study, animals are being exposed for 3 years to obtain support for the histopathological data and to augment information concerning the effects of nicotine on cardiopulmonary systems (Battelle Pacific Northwest Laboratories and Enviro Control Research Laboratories). Extensive pulmonary function tests are being performed in a chronic smoke exposure study (2 years) with dogs (Hazleton Laboratories America, Inc.). It is too early to discuss the results of these studies; however, the data are expected to increase our knowledge regarding the extent of functional impairment attributable to cigarette smoking.

In addition to studying pulmonary impairment, the effects of smoking on cardiovascular disease are being investigated. A 2-year study with dogs is being conducted to determine whether nicotine, carbon monoxide, and nitrogenous oxides accelerate the development of atherosclerosis (Hazleton Laboratories America, Inc.). Another animal model, the pigeon, is being used to further document the effect of nicotine and carbon monoxide on atherogenesis (Harvard University).

In an effort to simulate the human smoking pattern further, researchers have also investigated the feasibility of using a baboon inhalation model (Southwest Foundation for Research and Education). The necessary equipment and techniques have been developed, and animals have been trained to accept cigarette smoking. Carboxyhemoglobin levels and puff profiles indicate that baboon smoking patterns are similar to those of humans. Further development of this inhalation bioassay will provide an additional screen for selected less hazardous cigarettes.

In addition, new bioassay techniques have been devised to increase knowledge of the effects of cigarette smoke at specific cellular sites. An in vivo model for lung pellet implantation has been used to observe directly the carcinogenic effect of smoke condensate activity on lung tissue (Battelle Pacific Northwest Laboratories). In vitro bioassays have been

designed to assess smoke condensate effects on 1) growth and phagocytic activity in cultured cells and 2) ciliary kinetics and mucus transport in respiratory epithelium (Arthur D. Little, Inc.). Statistical correlation has been found between the concentration of smoke components and the following: potency for ciliatoxicity, inhibition of phagocytic activity, and potency for cytotoxicity.

Analysis of the experimental results discussed above under Chemical and Biochemical Analyses and Bioassay for Tumorigenic Activity indicates that cigarette materials can be manufactured to yield smoke with very low tumorigenic activity. Table 5 lists the properties that have been found to be important in the development of candidate less hazardous cigarettes. Knowledge of these characteristics, combined with data presented in Figure 6, strongly indicates that less hazardous smoking products can be made commercially available now. The collaborative research efforts discussed previously make such less hazardous products possible.

Table 5

Properties of Candidate Less Hazardous Cigarettes
as Determined from the Smoking and Health Program Activities

- Use of high-porosity paper as wrappers
- Large proportion of reconstituted tobacco sheet in the blend
- Use of tobacco extracted with water, hexane, or detergent
- Use of inert fillers as tobacco extenders
- Use of tobacco varieties from selected culture and curing methods
- Reduced tar content
- Use of filters and selected "best choice" smoke dilution devices
- Use of flavor additives to ensure acceptability

Individual Risk Analysis. To ensure complete achievement of Smoking and Health Program objectives, it is necessary to identify those individuals at high risk of developing tobacco-related diseases. Epidemiologic studies are presently investigating the relationship between tobacco-related diseases and smoking habits (American Health Foundation). Current data indicate the following:

- For those who have smoked for an extended number of years, there is a reduction in lung cancer risk when low- rather than high-tar cigarettes have been smoked for more than 10 years.

- For those who have smoked for an extended number of years, there is a reduction in larynx cancer risk when filter rather than nonfilter cigarettes have been smoked for more than 10 years.

- A relationship may exist between tobacco use and nonpulmonary cancers and other diseases (e.g., pancreatic and urinary tract cancers).

- Lung cancer risks are greater for lower educational and socioeconomic groups.

The extensive data base for the United States now covers eight cities and is still expanding. Further data concerning specific smoking habits, types of tobacco smoked, etc., are being gathered.

In addition, epidemiologic studies have been initiated in five Western European countries (Enviro Control, Inc.). Because cancer incidence and mortality rates in Europe are different from those in the United States, data on the relationship between smoking and cancer in these countries should increase present knowledge of cancer etiology. Cross-national comparisons of smoking patterns and behavior will be made with the American data. Knowledge of the causative factors involved in smoking-related diseases will facilitate the reduction of both incidence and mortality rates.

To gain additional knowledge regarding the smoking patterns of humans, attempts are being made to design a machine for investigating human dosimetry under normal smoking conditions (Arthur D. Little, Inc.).

Methods were also studied for the routine testing of susceptibility to tobacco-related diseases. One such test assays lymphocytes for carcinogen-induced aryl hydrocarbon hydroxylase (AHH) activity (Microbiological Associates, Inc.; North Texas State University). Laminar flow electrophoresis has been investigated to reduce variability in AHH analysis (North Texas State University). Two other projects involve the development of techniques for early detection of smoke-induced obstruction of small airways in the human lung (Johns Hopkins University; Veterans Administration Hospital, Miami, Florida).

Assisting Populations at Risk. Perhaps the most effective approach to assisting persons at high risk is help them achieve permanent smoking withdrawal. A project concerned with the development of an effective system for administering nicotine aerosol to individuals is currently in progress (American Health Foundation). Because nicotine may play a habituating role in smoking, it has been suggested that administering pure nicotine would facilitate smoking withdrawal. Work continues on developing an acceptable aerosol delivery system, with plans to test its effectiveness with volunteer subjects.

Smoking and Health Program

Contract Narratives

AMERICAN HEALTH FOUNDATION (SHP-74-104B)

Title: Clinical Trials for Pharmacological Approaches to Smoking Withdrawal

Contractor's Project Director: Dr. Alfred A. Smith

Project Officer (NCI): Dr. Gio B. Gori

Objective and Methodology: The objective of this project is to determine whether drug therapy, in conjunction with professional counseling, can be successfully used to assist human beings in withdrawing from cigarette smoking.

Agonists and antagonists, administered under clinical conditions, will be used to alter the patient's response to nicotine. Agonists will be given in aerosol form using a Babington nebulizer, and antagonists will be administered in pill form. Blood chemistry determinations will be used to verify withdrawal experience. ECG stress tests will be used to measure physiological changes, and counseling and questionnaires will be used to assess adverse drug reactions during the treatment. During this phase of the program, studies were undertaken with a modified Babington nebulizer to provide evidence of nicotine or cotinine uptake through inhalation of aerosols and, in addition, to establish clinical acceptability of the inhalation procedure.

Major Findings: Babington nebulizers were calibrated in the laboratory for dosage delivery using radioactive solutions and spirometry. The Babington nebulizer was found to be more useful than the DeVilbiss, which was less reproducible and produced some large clumps of particles.

Clinical trials were carried out with volunteer smokers and nonsmokers. Aerosol delivery of nicotine was refined, and effects on subjects were measured. Findings suggest that light smokers or nonsmokers are more sensitive to the vascular effect of nicotine than are regular users.

This project will continue with human subjects using several combinations of agents plus agents with placebo. It is expected that combinations of agonists and antagonists will substantially aid the patient in withdrawal from smoking.

Significance to Biomedical Research and the Program of the Institute: The objective of the Smoking and Health Program is to decrease the incidence and mortality of smoking-related diseases. This project approaches that aim by aiding in smoking cessation, as ex-smokers have reduced risks for tobacco-related diseases. The pharmacological approach will be directed to those persons who desire to stop smoking but have thus far been unsuccessful in doing so.

Date Contract Initiated: September 15, 1975

Current Annual Level: $50,000

AMERICAN HEALTH FOUNDATION (SHP-74-106)

Title: Evaluation of Carcinogenic Agents in Cigarette Smoke.
Part I - Epidemiological Evaluation of Carcinogenic Agents in Cigarette Smoke

Contractor's Project Director: Dr. Ernst L. Wynder

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The basic objective of the study is to monitor the use of the "less hazardous cigarette" among a hospital-based population and to determine whether the widespread reduction of cigarette tar yields has been translated into appreciably lower relative risk for certain cancers and other tobacco-related diseases. The continued goal of the research is to further document and refine the dose levels at which cigarettes (particularly filtered cigarettes, low in tar) exert the least detectable injurious effect on man's respiratory, upper alimentary, urinary, and cardiovascular systems. It is believed that documentation of such a level will aid in the prediction of future disease trends and will be of benefit in the identification and implementation of necessary and effective preventive measures.

Major Findings: As of February 1977, approximately 28,000 hospitalized individuals have been questioned by trained research interviewers at selected hospitals in New York, Los Angeles, San Francisco, Houston, Chicago, Miami, Birmingham, and Philadelphia. During the fiscal year 1976-1977, interviewing activities were curtailed in Houston and Miami. Negotiations are currently being conducted to gain access to patients in Omaha, Nebraska and Pittsburgh, Pennsylvania in order to document the smoking habits and disease trends in other industrial and Midwestern cities. The addition of these two cities to the study population, plus the possibility of other cooperating areas of the United States, will aid in making the data more representative of the entire country.

The study continues to demonstrate a reduced risk of cancer of the respiratory tract and oral cavity and, to a lesser extent, cancer of the esophagus and bladder among individuals smoking filtered cigarettes. There is a greater decrease in risk among younger age groups who have had a relatively shorter exposure to the smoke of high "tar," nonfiltered cigarettes than among older age groups. The sex ratio, especially for lung cancer (Kreyberg I), is declining, and this finding is consistent with the stabilizing rate of lung cancer among men and its increasing rate among women.

The thrust of the epidemiological evidence is that death rates are higher in smokers than in nonsmokers and increase with the degree of exposure to tobacco smoke. The chronic toxicity of tobacco smoke is reflected in the variety of diseases associated with its intake: 1) cancer of the lung, mouth, larynx, and bladder; 2) CHD, stroke, and aortic aneurysm; 3) COPD and emphysema; and 4) other diseases, including peptic ulcers and even accidents. To the extent that a specific disease relates to intensity of smoke exposure, results suggest that tobacco-related diseases predominate in males over females and in blacks over whites and that they similarly affect lower socioeconomic groups.

As evidenced from the large set of control data, tobacco usage in males is more common among those with lower levels of education and in the non-Jewish and non-white populations. Smoking cessation and use of low-tar cigarettes is significantly more prevalent in the white males of high socioeconomic status and among women. Interestingly, the data indicate that white males with higher levels of education consume slightly larger quantities of cigarettes per day than black, lower socioeconomic males. Across all races and educational levels, however, amount per day does not significantly vary between filter and nonfilter smokers.

Significance to Biomedical Research and the Program of the Institute: Determining and publicizing the hazards of smoking are still a major responsibility of the NCI. The American Health Foundation is one of the few institutions with an active history in pioneering and refining epidemiological methods in tobacco-related health research. Our staff has developed an entire systems approach, from hospital selection to interviewer training, computer analysis, and coordination of analysis of these data with the results of the laboratory investigations at the Naylor Dana Institute for Disease Prevention. Such a close interrelationship of disciplines is essential to fully develop epidemiological and experimental leads.

By exploiting this experience and our human resources, we have built a sound methodology for discriminating among various health effects resulting from varying tar/nicotine levels. Our research to date is clearly consistent with the other published studies (e.g., Hammond) and is geared ultimately towards developing dose-response relations quantitatively linked to tar/nicotine consumption.

Proposed Course: To continue data collection in the United States, with expansion of the study to additional hospitals to further balance the socioeconomic distribution of the patient population available for study.

Title: Evaluation of Carcinogenic Agents in Cigarette Smoke.
Part II - Laboratory Studies

Contractor's Project Director: Dr. Dietrich Hoffmann

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To isolate and to identify tumor initiators, tumor promoters, cocarcinogens, and organ-specific carcinogens and to explore their mode of formation and reduction in tobacco and tobacco smoke. It is a major objective of this research program to define the less hazardous cigarette.

Major Findings: The presence in tobacco smoke and in chewing tobacco of tobacco-specific organotropic carcinogens derived from alkaloids was substantiated. It was shown that the predominant N'-nitrosonornicotine (NNN) is formed from nicotine during curing of tobacco, as well as by pyrosynthesis during smoking of cigarettes. About 50% of NNN in tobacco smoke is contributed by direct transfer from tobacco, whereas the remainder is generated during smoking. NNN induces laryngeal and nasopharyngeal carcinomas in rats, lung adenomas in mice, and tracheal-bronchial tumors in Syrian Golden hamsters. Another tobacco-specific carcinogenic nitrosamine (NNK) derives from oxidative nitrosation of nicotine and is a more potent carcinogen than NNN in strain A mice. These tobacco-specific carcinogens are also formed during chewing, and preliminary analytical investigations show that they are present in the blood serum of smokers and are related to nicotine uptake. MH-30 (sucker-growth inhibitor) treatment of tobacco leads to nitrosodiethanol formation (60 ng/cigarette).

Volatile nitrosamines (VNA) were detected and quantitated in tobacco, its main- and sidestream smoke, with the aid of the Thermal Energy Analyzer (TEA). The detection limit is 50 pg per cigarette. Major precursors for these compounds were nitrate and proteins in tobacco. Presently available filter tips are capable of removing 70 to 80% of the VNA from the mainstream smoke. Depending on the airflow rate around the burning cone of a tobacco product, the sidestream smoke levels of VNA can be several hundredfold above those in the mainstream smoke.

A rapid and highly sensitive method for the determination of hydrogen cyanide and cyanogen (inhibitors of the lung clearance mechanism) in tobacco smoke was developed. The quantitative assessment of cocarcinogenic catechol and alkylcatechols was established. Smoke profile analyses have been developed for quinones, and for aromatic amines, several of which are known bladder carcinogens.

Combined chemical and bioassay studies towards the identification of co-carcinogens in the neutral and weakly acidic portions of smoke particulates

progressed. New findings were that the 3- and 4-ring aromatic hydrocarbon fractions ("phenanthrene fractions") and the PAH subfractions contain the major neutral cocarcinogens. The activities observed for these materials cannot be explained by the polycyclic hydrocarbons. Bioassays also revealed that several alkylated naphthalenes induce lymphomas in mice.

Short-term bioassays have indicated that those subfractions of the weakly acidic tobacco smoke condensate fractions that contain specific alkylated hydroxy- and dihydroxybenzenes are cocarcinogenic.

Significance to Biomedical Research and the Program of the Institute: The American Health Foundation has a coordinated research program on the epidemiology of tobacco-related cancers and on the identification and reduction of carcinogenic agents in tobacco smoke. This enables us to contribute to an understanding of the hazards involved in the use of tobacco products and continues to lead to a definition of the less hazardous cigarette, a major goal of the Division of Cancer Cause and Prevention of the NCI. The fact that more than 60 million Americans continue to smoke, despite great efforts to inform the public of the dangers of smoking, necessitates that the less hazardous cigarette remain one major goal of the American Health Foundation.

Proposed Course: The evaluation of the carcinogenic and cocarcinogenic potential of cigarette smoke constituents by chemical analytical identification, together with bioassays, will continue, with special emphasis on organ-specific carcinogens. Identification of tobacco carcinogens implicated in cancer of the bladder, kidneys, and pancreas in man and provisions for the reduction of such compounds will constitute main areas of research.

Date Contracts Initiated: February 24, 1970

Current Annual Level: $648,000

BATTELLE PACIFIC NORTHWEST LABORATORIES (NO1-CP3-3374)

Title: Research on Standardizing the Lung Pellet Implantation Technique in the Rat

Contractor's Project Director: Dr. Gerald E. Dagle

Project Officers (NCI): Dr. Thomas B. Owen
Dr. Mearl F. Stanton

Objective and Methodology: To evaluate a beeswax-tricaprylin-cigarette smoke condensate lung pellet implantation technique to standardize the technique for the bioassay of selected cigarette smoke condensates.

Osborne-Mendel rats were implanted with lung pellets through a thoracotomy incision with two different cigarette smoke condensates (CSC), with each CSC having three concentrations (1:50, 1:10, and 1:2 CSC to beeswax-

tricaprylin vehicle) and each concentration having three pellet sizes (0.05, 0.1, and 0.2 ml). Rats were sacrificed at 10, 30, 60, 90, and 120 weeks to study the effect of the release of CSC into surrounding lung parenchyma. Sprague-Dawley rats were also implanted to compare another strain of rat.

Major Findings: Invasive and metastasizing epidermoid carcinomas of the lung, similar to smoking-related lung cancer in man, were induced with this procedure. A 42% prevalence of pulmonary carcinomas was present in the highest dosage group, which had 67 mg of CSC exposing approximately 1.65 cm^2 of bronchiolar epithelium. A dose relationship was observed in the Osborne-Mendel rats, with the more concentrated CSC producing a greater number of pulmonary carcinomas. Squamous metaplasia associated with the injection site preceded the appearance of the carcinomas and was more severe in the larger pellets having more concentrated CSC. At the single CSC dose level and pellet size used in Sprague-Dawley rats, the incidence of pulmonary tumors was similar to that observed in the Osborne-Mendel rats. There was no difference in incidence of pulmonary carcinomas using CSC containing high or low concentrations of nicotine.

Significance to Biomedical Research and the Program of the Institute: The induction of epidermoid carcinoma in the lungs of rats by implantation of relatively small amounts of CSC in the lungs suggests that the lung pellet implantation technique can be used for routine bioassay of the carcinogenicity of cigarette smoke condensate in the development of a less hazardous cigarette. The technique offers an advantage over the mouse skin painting method, since the lung is the target tissue. Also the technique is less expensive than repeated intratracheal injections or inhalation studies and has the assurance of a high incidence of lung carcinomas to allow statistical comparison among groups of animals and different substances. The carcinogenic potential of different tobaccos, tobacco fractions, tobacco substitutes, and their combinations can be evaluated and compared. The technique also provides a model for the study of the mechanisms of pulmonary carcinogenesis.

Proposed Course: The contract expired December 31, 1976.

Date Contract Initiated: June 30, 1973

Current Annual Level: Was $25,000

BATTELLE PACIFIC NORTHWEST LABORATORIES (NO1-CP4-3315)

Title: Inhalation Bioassay of Cigarette Smoke in Dogs: High and Low Nicotine Content

Contractor's Project Director: Dr. Gary M. Zwicker

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: (1) To develop the technique and procedures for using the beagle dog bioassay model to determine relationships of modified tobacco character-

istics and cigarette composition to pathological effects. 2) To evaluate the beagle dog bioassay model comparing the effects of cigarette smoke from high and low nicotine cigarettes.

Major Findings: The project was divided into two parts. The first year, Phase I, was directed toward establishing the techniques and procedures to administer cigarette smoke to beagle dogs and application of quantitative measurements for characterization of smoke exposure and related biological effects.

During this reporting period, beginning July 1, 1976, Phase I was completed with a final report submitted to the NCI, November 12, 1976. Phase II was also initiated with expansion of capability, equipment and technicians to expose three groups of 60 dogs each to smoke from experimental cigarettes containing high, low, and extremely low concentrations of nicotine. A group of 40 sham-exposed dogs served as controls. Exposures were conducted for a total of 200 days for each group, 7 days per week and 12 cigarettes per dog each day. The following tasks of Phase II were conducted at BNW: (1) Six dogs/group were killed at 100 days exposure and 200 days exposure; (2) quality of cigarettes and of smoke exposures was monitored; (3) dosimetry measurements were made; (4) biomedical measurements were made; (5) histopathology was performed on all dogs that died or were killed; and (6) pulmonary morphometry was performed on all dogs that were sacrificed. Exposures were discontinued at BNW on November 13, 1976 at which time the dogs were transferred to Enviro Control, Inc. (ECI). A final report in tabular form, without statistical analysis or evaluation of the results, was submitted to the NCI on March 31, 1977 according to directions given by Dr. T. Owen, the NCI Project Officer.

Significance to Biomedical Research and the Program of the Institute: One of the goals of the NCI Smoking and Health Program is to develop a less hazardous cigarette. Bioassay models are necessary to evaluate the effect of modified tobacco or cigarette composition. The project was to develop the techniques and procedures for a beagle dog bioassay model which could be used to compare the biological effects from cigarettes of different compositions.

Proposed Course: The contract was terminated March 31, 1977.

Date Contract Initiated: June 30, 1974

Current Annual Level: $707,000

BATTELLE PACIFIC NORTHWEST LABORATORIES (SHP-75-121)

Title: Inhalation Bioassay of Cigarette Smoke in Rats

Contractor's Project Director: Dr. Alfred P. Wehner

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The objectives of this project are to set up an inhalation bioassay of cigarette smoke in rats; to validate experimental procedures and techniques; to evaluate this bioassay system; and to assess the relative hazard of different types of cigarettes by chronically exposing groups of rats to their smoke, using cardiorespiratory functions, body weight, and histopathologic changes as criteria.

Major Findings (as of 4/1/77, after 10 months of exposure): *Chronic Physiological Effects of Cigarette Smoke (measured while the animals were not exposed to smoke)* - The sham-exposed animals (female Fischer rats) had a significantly ($P < 0.05$) higher heart rate and higher respiratory frequency than the smoke-exposed animals. When respiratory frequency values were corrected for individual body weight differences, only animals exposed to smoke from code 27 cigarettes were significantly different from the sham-exposed controls. The tidal volume of the sham-exposed animals was significantly lower than that of the three smoke-exposed groups. Code 27 animals had a significantly lower heart rate and lower respiratory frequency than the code 13 and code 32 animals. When respiratory frequency values were corrected for individual body weight differences, there were no significant differences between all three smoke-exposed groups.

The mean body weights (MBW's) of the shelf controls are much higher than those of all other groups, including the sham-exposed controls. The MBW's of the sham-exposed animals are significantly higher than those of the smoke-exposed animals.

Acute Physiological Effects of Cigarette Smoke (measured while the animals were exposed to smoke) - Means for the number of breaths, tidal volume, and total inhaled volume were similar among the smoke-exposed gorups. Mean values for all smoke-exposed groups were significantly lower than those of the sham-exposed controls for all respiratory parameters measured.

A marked reduction in heart rate during smoke exposure occurred in animals exposed to smoke from all three cigarette codes, whereas little change was observed in sham-exposed controls. The degree of bradycardia was similar in the three smoke-exposed groups.

Significance to Biomedical Research and the Program of the Institute: The results of this study can be compared with other bioassay methods as a measure of the tumorigenic hazards of cigarette smoke inhalation. The study will assess the relative degree of hazard of the three cigarette types, not previously tested by inhalation, for pulmonary and cardiovascular effects, as well as for lung and other cancer. This study will evaluate the rat bioassay system for assessing the effects of cigarette smoke with regard to the NCI programmatic objectives to develop less hazardous cigarettes. It will develop and refine the technology and procedures for long-term exposure of rats to cigarette smoke for future inhalation bioassays of cigarette smoke.

Proposed Course: The experimental groups will continue to be exposed, in Maddox-ORNL smoking machines, to smoke from three cigarette codes (eight

exposures/16-hour day, 7 days/week, for up to 24 months; dose = 560 puffs/week). Physiological measurements, dosimetry, and quality control measurements will continue according to the experimental protocol.

The first serial sacrifice of 10 rats/group after 12 months of exposure is scheduled for May, to be preceded by an extended series of function measurements.

Date Contract Initiated: June 30, 1974

Current Annual Level: $660,000

ENVIRO CONTROL, INC. (NO1-CP5-5666)

Title: Smoking and Health Prime Contract

Contractor's Project Director: Dr. Cornelius J. Lynch

Project Officer (NCI): Dr. Thomas B. Owen

Objective and Activities: The objective of this contract is to provide a wide range of scientific and administrative services to the Smoking and Health Program. Because this program involves a complex host of resources, particular demands are made for fiscal, technical, administrative, and managerial skills.

As the Prime Contractor, Enviro Control is responsible for the successful execution of subcontracts and also participates in Smoking and Health Program decisions by performing analyses, evaluating alternatives, and recommending appropriate courses of action. Two distinct types of activities are involved.

First, as the Prime Contractor, Enviro Control manages individual subcontracts and groups of projects and is responsible for accumulation and analysis of the resulting data. Preparation of project plans, evaluation of proposals, and negotiation and award of new subcontracts are additional activities of the Prime Contractor. New research concepts are continually being sought and developed for future use in the Smoking and Health Program.

A second activity under this contract involves budgetary management and overall coordination of the program, which include experimental cigarette procurement, contract advisory support to the Director of the Smoking and Health Program, administrative support for the Tobacco Working Group, and the scheduling and conducting of workshops, conferences, and contractor meetings. In addition to the above, the Inhalation Bioassay of Cigarette Smoke in Dogs (Subcontract NO1-CP4-3315) has recently been transferred to this contract by NCI.

Major Findings: This contract has been primarily for support and management, not research per se. Accomplishments this year consist of approximately 50

reports, memoranda, and analyses of various aspects of smoking and health, including statistical and inferential analysis of laboratory, epidemiologic, and literature data and organization, support, and follow-up documentation for a dozen workshops, conferences, and contractor meetings. More than 12 new contracts have been established, with significant cost and information benefits. The contracts have been negotiated as integrated portions of a coordinated whole. The inhalation bioassay project has not been part of this contract long enough for researchers to report any findings at this time.

Significance to Biomedical Research and the Program of the Institute: The significance of these activities is inseparable from that of the entire Smoking and Health Program. Each of the activities performed is essential to the integrity and success of the program. Enviro Control serves the interests of the Smoking and Health Program by carrying out the activities under the direction of the NCI (technical and contracts branches).

Date Contract Initiated: January 1, 1975

Current Annual Level: $1,225,000

ENVIRO CONTROL RESEARCH LABORATORIES (NO1-CP5-5666-SA12)

Title: Inhalation Bioassay of Cigarette Smoke in Dogs: High- and Low-Nicotine Content

Contractor's Project Director: Dr. Allan G. Manus

Project Officer (NCI): Dr. Thomas B. Owen

Objective and Methodology: The objective of this project is to develop the techniques necessary for the use of a beagle bioassay model.

The effects of high- and low-nicotine cigarettes are tested using tracheostomized dogs routinely exposed to cigarette smoke. Pulmonary and cardiovascular function tests and routine chemical analyses of blood are performed. Histopathology and morphometry are performed on animal tissue.

Major Findings: During the first phase of the study (NO1-CP4-3315), techniques were developed for making cardiopulmonary function measurements. Initial histopathological data indicated definite physiological changes resulting from chronic exposure to smoke. Groups of dogs smoking as few as six cigarettes per day, 5 days a week, had apparent lung damage after 1 year of exposure.

In addition, all data were examined to determine the extent of pulmonary changes, including emphysema and cardiovascular system evaluation.

Significance to Biomedical Research and the Program of the Institute: One of the major goals of the Smoking and Health Program is to develop a less hazardous cigarette. To attain this goal, researchers must use several

methods (including bioassay models) for evaluating the effects of modified tobacco and cigarette composition. This project is a continuation of research to refine the techniques and procedures for a beagle dog bioassay model. The model is to be used to compare the biological effects of various experimental cigarettes, with current emphasis on cigarettes differing in nicotine yields.

Date Contract Initiated: September 1, 1976

Current Annual Level: $1,614,000

ENVIRO CONTROL, INC. (SHP-75-120)

Title: An International Epidemiologic Study of the Relationship Between Lung Cancer and Cigarette Smoking

Contractor's Project Directors: Dr. C. Gillis (Scotland)
Dr. R. Schmähl (Germany)
Dr. R. Flamant (France)
Dr. M. Kunze (Austria)
Dr. A. Caputo (Italy)
Dr. F. Berrino (Italy)

Project Officer (NCI): Dr. Gio B. Gori

Objectives and Methodology: The purpose of the study is to determine parameters of tobacco usage in Europe and to investigate the relationship of these parameters to lung cancer incidence and mortality rates.

A retrospective case/control design will be utilized. Subjects will be selected from several hospitals in each country, and tobacco usage information will be obtained through interviews. Through data analyses, researchers will investigate the relationships of age, duration of smoking, frequency of smoking, type of tobacco smoked, form of smoke exposure (i.e., cigarette, cigar, pipe, snuff), occupation, and drinking habits to lung cancer incidence and mortality.

Major Findings: The project was started in September of 1976; therefore, there are no findings to report at this time.

Significance to Biomedical Research and the Program of the Institute: The data obtained in this study will be compared with data being collected in the United States and Cuba. Such comparisons will elucidate the etiology of lung cancer, which will enable NCI to design the methodology for reducing the carcinogenic potential of tobacco usage.

Date Contract Initiated: September 1, 1976

Current Annual Level: $250,000

ERDA-OAK RIDGE NATIONAL LABORATORY (YO1-CP4-0206)

Title: Cigarette Smoke Bioassay Development

Contractor's Project Director: Dr. Paul Nettesheim

Project Officer (NCI): Dr. Gio B. Gori

Objective: To develop new methods for assessing the biological impact of cigarette smoke.

Major Findings: The chronic cigarette tobacco smoke inhalation studies with rats have two exposure categories: lifelong exposure and sacrifice after specified lengths of exposure. The first exposures were begun on January 28, 1974, and additional groups of rats have been added to provide 120 rats exposed to 7 cigarettes/day, 40 rats exposed to 10 cigarettes/day, 65 container controls, and 90 age controls. All exposures were intermittent (30/30 second smoke/fresh air cycle) to nominal 10% smoke. Groups of approximately 10 rats from each category have been sacrificed after 12, 18, and 24 months of exposure to 1) follow the time course of lesion development under more rigidly controlled conditions of death and tissue fixation than is possible with spontaneous deaths and 2) examine possible physiological and biochemical indices of the impact of smoke exposure. The above serial sacrifices have been completed; the tissue and data derived from them are being evaluated. The remaining animals are being kept for life in a survival study. Exposure of these rats was stopped in January 1977. About 20% of the animals remained at this time, and the advantage of a prolonged lifespan in allowing further lesion development outweighed the relatively small number of additional exposures that could be performed before death of the animals.

Mortality has been very favorable compared to other, similar studies in the past despite approximately 1.75 mg of cigarette particulates deposited in the lung daily (0.25 mg/cigarette X 7 cigarettes/day). Of the animals receiving 10 cigarettes/day, 50% were dead after 81 weeks of exposure as compared to 104 weeks for the 7 cigarette/day groups. The mortality curve for age controls was similar to that for the 7 cigarette/day animals. Mortality was most favorable in the container controls (50% dead after 120 weeks). Mortality rates for 7 and 10 cigarette/day groups were very similar when referenced to the total number of cigarette exposures at death.

Deaths during the chronic exposures have resulted from a variety of causes, many expected in a colony of aging rats (such as pituitary tumors and lymphoma). Several lesions attributed to smoke exposure have been observed in the respiratory tract. Scattered hyperplastic-metaplastic areas of tracheal epithelium were observed, some with capillaries among the epithelial cells. Lungs had scattered dark foci and white subpleural nodules on gross observation, particularly on distal surfaces of lower lobes. In and around these focal lesions are collections of pigmented macrophages, disrupted and dilated alveoli, an increase in fibrous and fibrillar elements of alveolar walls, proliferation of histocytic and fibroblastic elements, and endothelial hyperplasia.

Respiratory and cardiovascular tissue from spontaneously dying rats is being evaluated using routine H & E sections. Respiratory tissues from animals in the serial sacrifice are being treated similarly but with special stains, morphometric analysis, and scanning electron microscopic evaluation in addition. Transmission electron microscopy of selected tissue is being used to substantiate light microscopic observations.

Measurements made during the serial sacrifice included electrocardiograms, respiratory minute volume, total static compliance, frequency dependence of dynamic compliance, a forced deflation maneuver, blood cell counts, osmotic fragility of erythrocytes, and a series of biochemical indicators in plasma. Data have been entered into a computer and are currently being evaluated statistically.

Several acute ancillary experiments have also been performed. Nicotine was found to be a possible contributor to the development of tolerance with successive exposures. In rats after 3 days of exposure, blood carboxyhemoglobin was about 1% at the start of the 4th day's exposure to 7 cigarettes and reached a peak of approximately 10%. A maximum of about 17% was observed after 7 cigarettes were given during 3.5 hours rather than over a full 8-hour day used above. Nonetheless, body weight reduction in rats exposed to 7 cigarettes during 3.5 hours/day for 20 weeks was considerably less than that observed with exposures over 8 hours. The daily schedule of animal handling may significantly influence exposure-related changes in body weight.

Acute toxicity (mortality) and respiratory deposition of smoke particles were investigated in relation to smoke concentration (nominal 10 or 20%) and exposure time per puff (30 or 40 seconds). Acute toxicity, blood COHb, and pulmonary deposition increased in the order: 10% and 30 seconds, 10% and 40 seconds, 20% and 30 seconds, 20% and 40 seconds. These data indicated that exposure duration per puff might be increased from 30 to 40 seconds (with 10% smoke) without untoward increase in toxicity. A regional distribution of pulmonary deposition of particulates was observed under all exposure conditions, the right superior lobe having the highest TPM per gram wet weight and the right inferior lobe the lowest.

A low level of effort has been maintained on the tracheal transplant model for exposing respiratory epithelium to large doses of tobacco smoke without significant systemic toxicity. Transplants are currently being exposed by this method.

Significance to Biomedical Research and the Program of the Institute: The NCI has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps that might be taken to reduce this potential. Bioassay studies provide methods that will allow a more reliable definition of the relative biological activities of products under test.

Proposed Course: To complete tissue processing and evaluation and to finalize all data processing for assessment of the usefulness of this bioassay model.

Date Interagency Cooperative Agreement Initiated: July 1971

Current Annual Level: $184,000

ERDA-OAK RIDGE NATIONAL LABORATORY (Y01-CP6-0206)

Title: Collection, Separation, and Elucidation of the Components of Cigarette Smoke and Smoke Condensate. Part I - Chemical Characterization of Cigarette Smoke. Part II - Inhalation Bioassay Monitoring

Contractor's Project Directors: Dr. Michael R. Guerin
Part I - Dr. W. H. Griest
Part II - Dr. R. A. Jenkins

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Part I - To provide a maximum chemical characterization of the cigarette smokes undergoing biological testing by other NCI contractors.

Part II - To develop and implement procedures for exposure dose documentation, thus enabling more cost-effective interlaboratory comparison of inhalation bioassay studies.

Major Findings: *Part I* - The smoke and condensate analyses for the approximately 44 variants of the fourth experimental series are nearly complete. Twenty-eight routine protocol constituents were measured in four batches of condensate and two analytical runs of cigarettes. Non-protocol multicomponent profiling methods exist for measurement of gross TPM or condensate composition, phenolics, carboxylic acids, polynuclear aromatic hydrocarbons, polyglycols, terpenoids, gas-phase volatile organics, and sulfur compounds. Procedures have also been developed for determination of methyl chrysenes, alkyl naphthylenes, alpha emitters, alkaloids, and trace metals. At the request of the Project Officer, approximately five special studies including the following have been carried out: chemical characterization of 10 foreign and 17 domestic commercial brands of cigarettes, chemical evaluation of 24 codes of condensates regenerated from Series I-III variants for the sebaceous gland suppression bioassay test, and evaluation of a specific benzo[a]pyrene antibody as a selective filtration device.

Accomplishments beyond the results of the characterization of the primary experimental variants include 1) the development of an improved cigarette smoke gas-phase collection procedure for CO/CO_2 analysis, 2) development of wall-coated glass capillary columns for improved gas chromatographic resolution of alkyl naphthylenes and other PAH's, 3) tentative mass spectral identification of the constituents of a tobacco smoke PAH isolate, and 4) improvement of the method for measurement of polonium-210 in smoke condensate and the application of the method to condensates from nine commercial domestic brands. Results from the special studies suggested that quantitative

differences in smoke composition exist between different batches of some commercial brands, and that the condensates regenerated for the sebaceous gland suppression bioassay were quantitatively different from the original Series I-III condensates. Condensate batch generation size and cigarette aging are suggested as possible causative factors for the latter. Initial evaluation of the benzo[a]pyrene antibody did not indicate a selective filtration attributable to the antibody itself.

Part II - Protocols for the monitoring function have been formulated, and periodic working site visits to all of the laboratories conducting chronic inhalation exposures for NCI are being made. Machine puffing characteristics are measured to assess machine performance under chronic exposure conditions. Smoke samples are obtained to enable estimation of the dose that the animals receive and to determine the extent to which certain smoking and environmental factors can contribute to the variation in that dose. Recently, a more detailed chemical characterization of the smoke offered the animals has been emphasized.

Specifically, on-site monitoring has provided evidence for the existence of two distinct nicotine dose groups in one exposure study. Some preliminary evidence suggests that the dog exposure devices may somewhat alter the chemical composition of particulate phase of the smoke being offered the animals. Detailed examination of site visit data has indicated that, in one study, the primary contributor to dose variation has been a problem with routine exposure device maintenance, whereas in another study, variation of cigarette nicotine content is an important contributor to dose variation.

Other studies in support of the inhalation bioassays have provided the basis for recommendations to NCI/Enviro Control concerning the effective use of exposure devices in future inhalation studies. In addition, some preliminary investigations have suggested that, although there are machine-related differences in smoke aerosol particle size, these differences are probably small. Dosimetry consultation and services using radiolabeled smokes continue to be provided to NCI contractors. An instrumental monitoring device, based on light scattering of smoke particles, is being constructed and should provide more accurate estimates of the amount of smoke actually inhaled by the animal.

Significance to Biomedical Research and the Program of the Institute: The NCI has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps that might be taken to reduce this potential. Chemical studies provide the methods and data by which smoke constituents may be related to biological activity. The findings have great importance to other areas of carcinogenesis, e.g., occupational and environmental.

Proposed Course: *Part I* - To devise methods for the collection and analysis of smoke offered to animal models by inhalation bioassay exposure devices, to provide a chemical definition of the potential exposure. Also to respond rapidly to short-term special studies and requests.

Part II - To continue to provide data and methodology for dose documentation, to enable more cost-effective interlaboratory comparison of inhalation bioassay studies.

Date Interagency Cooperative Agreement Initiated: Part I - November 1969
Part II - September 1975

Current Annual Level: Part I - $188,000
Part II - $376,000

HARVARD UNIVERSITY (BETH ISRAEL HOSPITAL) (SHP-75-111)

Title: Inhalation Bioassay of Cigarette Smoke in Pigeons: Effect of Nicotine and Carbon Monoxide on Atherogenesis

Contractor's Project Officer: Dr. Gary L. Huber

Project Officer (NCI): Dr. Thomas B. Owen

Objective and Methodology: The objective of the investigation is to conduct a bioassay of cigarette smoke in pigeons.

- To conduct pigeon inhalation bioassays that will advance knowledge of the specific roles of nicotine and carbon monoxide in cigarette smoke in atherogenesis.
- To make qualitative and quantitative observations relating to the etiology and pathogenesis of smoke enhancement of this disease.
- To determine reasonable time limits for pigeon inhalation studies as a model for screening candidate less hazardous cigarettes.

Major Findings: None to date.

Significance to Biomedical Research and the Program of the Institute: The purpose of this project is to study the effect of cigarette smoke on enhancing the development of atherosclerosis and coronary heart disease in an experimental animal model, and to determine the feasibility of pigeons as a pre-screening model for candidate less hazardous cigarettes. The pigeon has been selected as the most suitable model for this study. Various strains do exhibit some degree of genetic purity and selected strains do have an increased susceptibility to the development of atherosclerotic lesions.

Date Contract Initiated: June 1, 1977

Current Annual Level: $88,000

HAZLETON LABORATORIES (SHP-74-101)

Title: Mouse Dermal Bioassay of Cigarette Smoke Condensates

Contractor's Project Director: Mr. James L. Gargus

Project Officer (NCI): Dr. Gio B. Gori

Objective and Methodology: To provide a standardized bioassay for the determination of the carcinogenicity of cigarette smoke condensates by repeated dermal applications on mice.

Whole-smoke condensates are supplied by Meloy Laboratories under NCI contract. The experimental variables include tobaccos of different origins; methods of cultivation, including fertilization, suckering agents, and soils; curing and aging methods; blends of tobacco plant parts such as leaves, stalks, and stems; reconstituted tobacco; cutting of tobacco; cigarette paper; and filters.

Female ICR mice are treated six times weekly with dermal applications of the whole-smoke condensates dissolved in acetone. Skin tumors are recorded and measured. All animals are necropsied, and tissues are fixed and preserved for histopathological examinations. Data on each animal are entered in a computer once monthly. Computations and printed reports from DCRT provide monitoring data for continuous evaluation of the experimental results.

Major Findings: The mouse dermal bioassays of the first, second, and third series of condensates have been completed. Results of these studies have been published in reports by the NCI.

The fourth series of 40 smoke condensates will complete the treatment period (18 months) in May 1977. Following necropsy of these mice, the tumor-bearing skin tissues will be sectioned, stained, and examined by the pathologist. The results will be entered in the computer file.

Significance to Biomedical Research and the Program of the Institute: The comparative bioassay results, along with other monitoring bioassays and chemical determinations, will permit evaluation of the biological effects of the variables in the different smoke condensates.

These comparative evaluations will provide data for testing and developing less hazardous cigarette formulations.

Proposed Course: Treatments and observations of mice in the fourth series of condensates will be continued. At 18 months, these mice will be necropsied and skin tissues will be examined by a pathologist.

Date Contract Initiated: March 1, 1974

Current Annual Level: $345,000

HAZLETON LABORATORIES AMERICA, INC. (SHP-75-112)

Title: Inhalation Bioassay of Cigarette Smoke in Dogs: Effects of Nicotine, Carbon Monoxide, and Nitrogen Oxides on Atherogenesis

Contractor's Project Director: Dr. William B. Coate

Project Officer (NCI): Dr. Thomas B. Owen

Objective and Methodology: The objective of this study is to conduct dog inhalation bioassays that will advance knowledge of the specific roles of nicotine, carbon monoxide and nitrogen oxides in atherogenesis, and to make qualitative and quantitative observations relating to the etiology and pathogenesis of smoke-enhancement of this disease.

Major Findings: None to date.

Significance to Biomedical Research and the Program of the Institute: A major objective of the Smoking and Health Program is the development of less hazardous cigarettes. To aid in attaining this objective, the purpose of this research is to assess the relationship of nicotine, carbon monoxide, and nitrogen oxides in cigarette smoke with the synergistic effect between these smoke components and an atherogenic diet on the cardiovascular system.

Date Contract Initiated: June 7, 1977

Current Annual Level: $75,000

HAZLETON LABORATORIES (SHP-75-117)

Title: Respiratory Impariment in Beagles Exposed to Cigarette Smoke

Contractor's Project Director: Dr. William B. Coate

Project Officer (NCI): Dr. Thomas B. Owen

Objective and Methodology: To evaluate four types of cigarettes (code numbers 13, 32, 79, and 90) in terms of respiratory impairment caused by chronic smoking.

Groups of 40 male beagle dogs began smoking in December 1975. Cigarettes are puffed by the A.D. Little Mark 2 smoking machine, and the smoke is inhaled through a cuffed tracheostomy tube. Ten cigarettes are smoked daily, 6 days per week. Sham smokers and tracheostomy controls are included. All dogs are routinely tested for changes in pulmonary function, for COHB, and for plasma nicotine levels. Serial sacrifices are being made for detailed pulmonary histopathology. Hemostasis is being evaluated at sacrifices.

Major Findings: After 15 months of smoking, no smoking-related changes have been observed in closing volume, DL_{CO}, distribution of ventilation, dynamic or static compliance, forced expiratory flow at low lung volumes ($MEF_{.85}$),

RV, ERV, FRC, or TLC. No changes in clotting or platelet aggregation have been observed. COHb and plasma nicotine levels indicated that heavy smoke inhalation is occurring with all cigarette types. Increasingly severe pulmonary morphological changes have been seen in smoking dogs after 3, 6, 9, and 12 months. After 9 and 12 months, the microscopic alterations in the trachea were characterized by moderate to marked squamous metaplasia, marked loss of goblet cells, moderate to marked basal cell hyperplasia, and tracheal gland hypertrophy. In the lobar and segmental bronchi, moderate to moderately severe basal cell hyperplasia and marked loss of goblet cells were the characteristic smoke-related microscopic alterations. In the lungs, numerous lipid granulomas and varying degrees of focal nonsuppurative alveolitis were the most constantly seen smoke-related microscopic alterations. The focal nonsuppurative alveolitis was characterized by thickening and infiltration of the alveolar septa, moderate to marked pneumonocyte hyperplasia and hypertrophy, and an infiltration of mononuclear macrophages and lymphocytes into the alveolar wall. In the areas of alveolitis, there were generally varying degrees of alveolar wall fibrosis. The numbers of alveolar macrophages were generally increased and were frequently found in association with the focal alveolitis. No differences between smoking groups were noted.

Significance to Biomedical Research and the Program of the Institute: This project is part of the program for the development of a less hazardous cigarette. The selection of the beagle and the particular smoking system employed was based on prior work in another laboratory, which appeared to demonstrate profound pulmonary pathology within 2 years of daily smoking by beagles. Control over dosimetry and ability to conduct meaningful pulmonary function tests routinely further recommend this animal model.

Proposed Course: The study will continue as planned through January 1978, at which time all smoking will be terminated and all animals will be sacrificed for histopathological examinations. Histopathology will be completed by June 30, 1978.

Date Contract Initiated: November 1, 1975

Current Annual Level: $604,000

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (SHP-76-130)

Title: Case Control Study of Lung Cancer in Cuban Women

Contractor's Project Director: Dr. Olga Joly

Project Officer (NCI): Dr. Gio B. Gori

Objectives and Methodology: Cuban women have the highest female mortality rate from lung cancer in the Western hemisphere. This study is intended to 1) test for the first time whether smoking of dark-tobacco cigarettes carries a risk different from that of light-tobacco cigarettes, 2) provide an opportunity to confirm the finding that there is an association between female lung cancer and certain pregnancy factors, and 3) provide an

opportunity to test whether the association between smoking and lung cancer in Cuban women is similar to that observed for females in other parts of the world.

The study is to be conducted as a case-control experiment, the cases being women hospitalized with lung cancer. Two controls per case are to be identified, one being a hospital control, the other being a neighborhood control.

Major Findings: This recently established study has not yet progressed to the point at which any significant findings are available.

Significance to Biomedical Research and the Program of the Institute: The results of this study will be compared to the results from both the domestic and European epidemiology studies to elucidate the etiology of lung cancer, thus enabling NCI to design methodology for reducing the carcinogenic potential of tobacco usage.

Date Contract Initiated: May 1, 1977

Current Annual Level: $18,000

JOHNS HOPKINS UNIVERSITY (SHP-74-103)

Title: Pulmonary Screening Tests for the Effects of Cigarette Smoking on Small Airways in the Human Lung

Contractor's Project Director: Dr. Harold A. Menkes

Project Officer (NCI): Dr. Gio B. Gori
(NHLI): Dr. Susan Hurd

Objectives: To develop a new method of analyzing forced expiration (moments analysis) for evaluating the effects of cigarette smoking on pulmonary function. We are comparing this test with other tests of lung function in studies designed to 1) evaluate the reproducibility of tests, 2) compare pulmonary function in young smokers and nonsmokers, 3) evaluate pulmonary function in approximately 4,000 subjects taking part in an epidemiology study, 4) test the acute effects of smoking, and 5) compare helium versus air spirograms in smokers and nonsmokers.

Major Findings: The major findings in the study, which are based on preliminary evaluation of the data, are as follows. 1) We are now able to describe and reproduce spirograms with the analysis of moments. The fidility of reproduction is very encouraging. Its application to forced expiration with air and helium is progressing. 2) Smokers appear to have more variability in their pulmonary function than nonsmokers. Whether this is related to varying responses to cigarettes or to hyperactivity to nonspecific agents, but induced by cigarettes, is unknown. 3) Although several pulmonary functions tests show differences between young smokers and nonsmokers,

including phase III of the single-breath nitrogen test and the FEV_1 with helium, we have been impressed by the difference in diffusing capacity. If problems associated with increased back pressure can be worked out, this suggests that vascular reactions may be occurring in the smokers. If so, our preoccupation with small airways dysfunction and "early" disease may be misleading. 4) In testing the acute reactions to cigarettes, we are impressed by the variability in responses. Although part of this variability may be related to "dose," it is likely that much of it derives from patient susceptibility. One of the major goals of our studies is to assess the importance of risk factors for COPD.

Significance to Biomedical Research and the Program of the Institute: In developing a less hazardous cigarette, the ultimate test model is the human subject. Before studies in humans can be initiated, however, two major tasks must be completed. First, the response to cigarettes has to be measured and its significance determined. Second, the determination of susceptible subjects must be made.

Proposed Course: Our studies should provide information about 1) tests of pulmonary function including moments analysis, closing volume, etc., which may be effective in screening for the effects of cigarettes and 2) relative susceptibility of subjects, including relatives of patients with COPD, allergic individuals, etc., which may determine who should be selected for testing cigarettes.

Date Contract Initiated: August 15, 1975

Current Annual Level: $150,000

ARTHUR D. LITTLE, INC. (SHP-74-105-SA1)

Title: A Coordinated Program on Biological Effects of Cigarette Smoke: Short-Term In Vitro Bioassays, Models of Neoplasia, and Other Aspects of Carcinogenesis

Contractor's Project Directors: Dr. Sam P. Battista
Dr. Mildred Broome

Project Officer (NCI): Dr. Gio B. Gori

Objectives: 1) To evaluate experimental NCI cigarettes in two bioassay test systems: a) growth of KB cells and b) dynamics of in vitro ciliary mucus transport activity. 2) To complete the study to determine whether chickens can be used as an effective inhalation model for carcinogenicity of cigarette smoke alone - and also in conjunction with impaired mucociliary clearance. 3) To investigate pigeons as an in vivo inhalation model for cigarette smoke bioassays. 4) To develop a bioassay model for selective exposure of segments of bronchus to doses of cigarette smoke and/or condensate. 5) To provide collaborative support for adaptation, modification, and demonstration of smoke delivery systems for use in other laboratories when requested.

Major Findings: In vitro bioassays for the fourth set of 39 experimental cigarettes have been completed for ciliatoxic and cytotoxic (KB cells) potency. Results show that three cigarettes had notable reductions in ciliatoxic potencies ($ED_{50} \geq$ than 8 puffs) and eight cigarettes had significantly reduced, but somewhat higher, potencies (ED_{50} 7.5 to 8 puffs). A notable reduction in cytotoxicity ($ID_{50} \leq 20$) was observed with two samples. Ranking and correlations of cigarettes for ciliatoxic vs. cytotoxicity potency and for individual smoke components vs. ciliatoxic and cytotoxic potencies will be carried out when analytical values are provided.

Neoplasia - The study to determine the reliability of the chicken as an inhalation model has been completed. Chickens with both intact and denuded tracheas were exposed 6 days/week to smoke from two cigarettes for periods of up to 3.5 years. In addition to smoke, a second group of animals was given diethylnitrosamine at 14-day intervals at the beginning of the study, and a third group given both smoke and DEN. Results to date show the following:

1) Carcinogenicity was not observed in the trachea by inhalation of cigarette smoke for 3.5 years, by administration of 10 doses of diethylnitrosamine at 14-day intervals, or by a combination of the two. The same is true if these agents are delivered to tracheal epithelium devoid of ciliary clearance and undergoing rapid regeneration from previous mechanical denudations. Application of smoke did appear to increase the incidence of lymphocytic proliferation.

2) The proportion of ciliated to nonciliated cells in tracheas of control animals and animals receiving smoke, DEN, or smoke along with DEN and sacrificed after 3.5 years of treatment was not significantly different. However, the number of goblet cells in the three treated groups was greater than in the controls.

3) No lung tumors were observed in animals that were sacrificed after 3.5 years. Lymphocytic proliferation and epithelial and smooth muscle hyperplasia of secondary and tertiary bronchi were the lesions of consequence observed in animals receiving DEN, smoke, or DEN and smoke.

4) Studies to adapt the Arthur D. Little Smoking System used for dogs and chickens to exposure of pigeons have been unsuccessful. Because of insufficient pressure and volume generated by the pigeon during normal respiration, it is not possible to operate presently available valving without the use of complex electromechanical assistors. In light of this problem, we recommend use of a continuous smoker to deliver smoke to a multiport chamber holding large numbers of animals.

Selective Exposure of Bronchi to Cigarette Smoke and Cigarette Smoke Condensate - During the past year, a number of different experimental approaches have been used to develop methodology for obtaining data on the carcinogenicity of smoke or smoke condensate applied directly to the bronchial epithelium, with a secondary goal of comparing the carcinogenicity of smoke condensate for mouse skin and bronchi. To achieve these goals, it was necessary to employ procedures that permit instillation of multiple doses

of native smoke, smoke condensate, and other agents as, for example, chemical initiators. One method of instillation utilizes bronchoscopy to expose the bronchus to smoke condensate and/or initiators. The procedure is carried out in anesthetized dogs. The second method utilizes the catheters previously implanted in the bronchi of rats and dogs to deliver the test material from the outside of the animal to the desired site in the lung. By using two catheters, this same method shows promise as a means of selectively exposing bronchi to large quantities of smoke. Smoke is introduced into one bronchus and removed from an adjacent bronchus. Implantation of catheters has been successfully carried out in both dog and rat, but only in the dog have long-term implants been successful. The present study consists of 13 dogs receiving bronchial exposure to one or more of the following: MNNG, MCA, DMBA, smoke, or smoke condensate. The delivery of these agents involves use of either bronchoscopy or bronchial catheter. In addition, with these procedures it is possible to visually observe and sample the exposed tissue for neoplastic and other pathologic changes instead of waiting until death or necropsy. Preliminary results show that, in one of two dogs, hyperplasia and metaplasia (possibly papilloma) occurred within 10 weeks after six treatments with MNNG. In another dog, a papilloma was observed 8 months after a single dose of MNNG delivered to the bronchus. After 20 weeks, two dogs given a single dose of initiator (DMBA), followed by daily doses of smoke condensate, have shown a narrowing of the bronchial lumen indicative of hyperplasia and metaplasia.

To augment future programs requiring continuous generation of fresh smoke, a reliable, low-cost smoking device was designed, and a working prototype was fabricated and tested. The concept involves a rotor having several cavities which rotates within a fixed member or stator containing three separate ports connected to a vacuum source, a cigarette, or an air pressure source. Smoking is accomplished by the rotation of the cavity as it moves from the vacuum port (to evacuate the air), to the cigarette port (to produce the puff), and finally to the air pressure port (to dilute the smoke and deliver it to the exposure system). The basic device has no valves and only one moving part.

Significance to Biomedical Research and the Program of the Institute: Identification of pathophysiological responses of various organ systems after *in vitro* exposure to smoke from various cigarette modifications is a vital consideration in the successful design of a less hazardous cigarette. The tasks in this contract consist of characterizing the potency of smoke from experimental cigarettes to inhibit cellular growth and function *in vitro* and carcinogenic potency of smoke *in vivo*.

Proposed Course: To perform bioassay procedures for ciliary inhibition and cytotoxicity for subsequent series of NCI experimental cigarettes. To continue development of methodology for selectively exposing bronchi to native cigarette smoke and smoke condensate daily with and without initiators. To complete development of the Arthur D. Little, Inc., rotary smoking machine for generating smoke continuously.

Date Contract Initiated: June 19, 1969

Current Annual Level: $126,000

ARTHUR D. LITTLE, INC. (SHP-75-110)

Title: Cigarette Dosimetry in Man: Development of Equipment and Techniques

Contractor's Project Director: Mr. H. Loeffler

Project Officer (NCI): Dr. Gio B. Gori

Objectivesand Methodology: The objective of this work is to develop a system for delivering measured amounts of smoke to human smokers. Additional objectives are that the system be minimally invasive, not interfere with normal smoking practice, and not intimidate the smoker. The transducer at the cigarette and associated real time display computation, and record-keeping systems shall be used in conjunction with a characteristic puff-describing profile for establishing desired smoke doses.

Major Findings: Contract initiated March 20, 1977 and is in Task 1 of Phase I at this report.

Significance to Biomedical Research and the Program of the Institute: The objective of the Smoking and Health Program is to decrease the incidence and mortality of smoking-related disease. A system to measure the delivery of smoke to an individual will assist in the evaluation studies of chemical markers for dosimetry independently of inhalation practices, help characterize the dose of the dose-response curve necessary to the epidemiology of smoking-related responses, and evaluate mechanism for curbing the extent of smoking, i.e., satiation parameters.

Date Contract Initiated: March 20, 1977

Current Annual Level: $85,000

ARTHUR D. LITTLE, INC. (SHP-74-116)

Title: Development of a Flavor System for Acceptability to Smokers of Candidate Less Hazardous Cigarettes

Contractor's Project Director: Mr. John Angeline

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this program is to identify and demonstrate a method for building acceptable flavor characteristics in a candidate reduced hazard cigarette. The means of developing these flavor characteristics should be through use of additive flavoring chemicals that will be incorporated into the cigarette by direct addition to the tobacco. The flavor agents employed will be defined chemical entities considered GRAS materials; they will be used in low concentrations and will be readily amenable to further inhalation toxicity studies.

To accomplish the objective of the program, the work was structured in five phases as follows:

Phase I	Screening and selection of pure chemicals, relying on flavor criteria to identify potential ingredients for flavoring mixtures.
Phase II	Building flavor mixtures to create levels of increased acceptability.
Phase III	Refinement of mixtures to build toward a predesigned model profile.
Phase IV	Evaluation of manufacturing feasibility.
Phase V	Preliminary investigations on the flavor effects of nicotine.

Major Findings: *Phase I* - Using cigarettes supplied by NCI (substrate IV-27), 277 organic chemicals were evaluated in cigarettes using specific screening criteria to measure their efficacy and usefulness. Of this group, 146 chemicals were selected as having potential for use in complex flavoring mixtures.

Each agent was evaluated using a protocol established by Arthur D. Little, Inc., which rated five characteristics of cigarette flavor including overall blend (amplitude), tobacco and woody aromatics, irritation, off-notes, and the presence or absence of a wet mouth feeling.

The 146 chemicals were categorized for potential usefulness, and the screening information was entered into an APL computer for retrieval and sorting. To be most cost-effective, the 62 chemicals with greatest potential use were studied first.

Phase II - During this phase approximately 375 flavor systems were compounded from chemicals categorized as useful in Phase I. Combinations ranging from two to six components were prepared and evaluated. At least 10 formulations from this group represented significant flavor change from the control substrate cigarette. Of these 10, 2 were considered sufficiently interesting to act as base products for flavor refinement in Phase III.

Phase III - Two six-component systems were used as bases for additional compounding, and approximately 30 seven- and eight-component systems were constructed. From this group, four seven-component systems and two eight-component systems were identified as meeting all of the predetermined flavor criteria for significant improvement in flavor acceptability of a reduced hazard cigarette.

Phase IV - Two formulations, one a four-component mixture and one a five-component mixture, were incorporated at different concentration levels in special sheets prepared by Peter Schweitzer Division of Kimberly-Clark

Corporation, under the direction of Dr. William Selke. After manufacture, the cigarettes were evaluated by the normal screening and flavor profile procedure. These evaluations indicated that, although the flavoring systems reacted differently on the different substrate, definite improvement in flavor was achieved through incorporation of flavorings by this conventional manufacturing process.

Phase V - A special set of experiments was conducted, using both free nicotine as the alkaloid and bound nicotine as the citrate as additives to a nicotine-free substrate, cigarette #63. This study demonstrated that nicotine, when added as an alkaloid at low concentrations (1 mg of added nicotine), contributes fullness, a decrease in charred wood aromatics, and an increased sense of wetness in the mouth. Nicotine, when added as the citrate, did not appear to produce positive flavor effects.

This research indicates that more work is needed in this area to establish the type and degree of flavor effect resulting from addition of bound and free nicotine in both tobacco and nontobacco substrates.

Significance to Biomedical Research and the Program of the Institute: The Smoking and Health Program seeks to identify the characteristics of cigarette construction associated with reduced biological activity. It also seeks, in this subcontract, to identify those characteristics of cigarette acceptability that can be reintroduced to an innocuous smoking matrix to produce consumer satisfaction, without substantial health hazard.

Date Contract Initiated: June 24, 1974

Current Annual Level: $44,000

MELOY LABORATORIES, INC. (SHP-75-118)

Title: Preparation and Analysis of Cigarette Smoke Condensate Samples

Contractor's Project Director: Dr. A. R. Patel

Project Officer (NCI): Dr. Thomas B. Owen

Objectives and Methodology: During a 1-year period, Meloy Laboratories will smoke over 3,000,000 cigarettes to produce the smoke condensate having, as nearly as possible, uniform composition for use in a number of bioassay programs to be carried out under contract in the Chemical Carcinogenesis/ Lung Cancer Task Force Program. This project is expected to terminate on June 24, 1977.

1) For each of the 40 types of cigarettes of the fourth series, the cigarettes were conditioned at 75 ± 2°F and 60 ± 5% relative humidity for at least 48 hours prior to being smoked on three manifold-type smoking machines furnished by the NCI. The smoke condensate collected in the glass trapping system was extracted with ACS-certified reagent-grade acetone. The acetone extract was concentrated under reduced pressure in a rotary evaporator at a temperature below 104°F, yielding a condensate with less

than 6% water. The residue was then adjusted by the addition of acetone and water (6%) to a composition of 250 or 500 mg of tar per ml of condensate. The smoke condensate was then transferred into narrow-mouth, amber glass 2-ounce bottles fitted with Teflon-lined screw caps. The condensate bottles were shipped weekly to Hazleton Laboratories in a dry ice container and, when required, to other contractors according to the instructions of the Project Officer. At least 250 g of dry condensate was produced from each of the 27 types of cigarettes, and 450 g of dry condensate was produced from each of the 13 types of cigarettes for an 8-week skin painting study.

Beginning February 15, 1976, Meloy Laboratories produced at least 100 g of cigarette smoke condensate from each of the 50 types of NCI experimental cigarettes for the sebaceous gland suppression test study to be carried out at Roswell Park Memorial Institute and the University of Kentucky Tobacco Research Institute. Since August 1976, Meloy Laboratories has been producing cigarette smoke condensate from four types of experimental cigarettes for a dermal bioassay project to be carried out at the American Health Foundation. About 18 kg of cigarette smoke condensate will be generated before the end of June 1977.

2) Meloy Laboratories carried out a quality control program of chemical analysis on all batches of cigarette smoke condensate, both to ensure uniformity and to characterize the product to some extent for the subsequent users. The following information was recorded on each batch:

a) Mean butt length
b) Total condensate yield and condensate yield per cigarette
c) Moisture content
d) Acetone content
e) Condensate pH
f) Nicotine content of the condensate and nicotine content per cigarette

3) During the 1-year period, Meloy Laboratories carried out the following analyses of each of the 40 types of fourth series cigarettes:

a) Nicotine in tobacco
b) Nitrate in tobacco
c) Reducing sugars in tobacco
d) Ash
e) Hexane solubles
f) Potassium and sodium in tobacco
g) Calcium and magnesium in tobacco
h) Neophytadiene in tobacco
i) Static burning rate of cigarettes
j) Average temperature of firezone at different butt lengths

Major Findings: This is a complex service contract, the scope of which is production of cigarette smoke condensate on schedule and which involves quality control of each batch of smoke condensate.

Significance to Biomedical Research and the Program of the Institute: This contract is designed to produce the cigarette smoke condensate required for the execution of three other projects that are funded under the Lung Cancer Task Force: 1) Skin Carcinogenesis Bioassay of Cigarette Smoke Condensate, 2) Chemical Analysis of Cigarette Smoke Condensate Samples, and 3) Bioassay of Cigarette Smoke Condensate by the Sebaceous Gland Suppression Test.

Proposed Course: Activities of this contract started in December 1975. Analytical work on the unsmoked tobacco from these experimental cigarettes is proceeding on schedule.

Date Contract Initiated: December 13, 1975

Current Annual Level: $212,000

PRINCETON UNIVERSITY (SHP-75-108)

Title: A Computer-Simulated Cigarette Model for Use in the Development of Less Hazardous Cigarettes

Contractor's Project Director: Dr. Martin Summerfield

Project Officer (NCI): Dr. Thomas B. Owen

Objectives and Methodology: The objective of this project is to develop a simplified mathematical model of cigarette combustion that will provide a rational description of the processes within a burning cigarette that convert tobacco into smoke and other chemical products. The ultimate aim is to provide an explicit link between cigarette design parameters and the resultant smoke composition via a computer program.

The first phase of the program will consider constant draw from ignition, including char oxidation and pyrolysis to tar. In Phase II, the thermophysical factors required to describe free burning will be added, and the Phase I model will be expanded to include CO, CO_2 and H_2O high-temperature reactions. Phase III will focus on three health-related species, i.e., CO, TPM, and nicotine, in a puff/relaxation cycle and includes experiments to test model predictions.

Major Findings: An initial model has been formulated and solved for the simplified situation of continuous draw from ignition. The model assumes a one-dimensional geometry for the entire cigarette and reduces the combustion chemistry to two overall reactions, tobacco pyrolysis and char oxidation. These reactions have been characterized kinetically and thermally using thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). Model predictions of burning rate and pressure drop vs. flow rate and ambient oxygen level compare quite favorably with results from analogous steady-draw experiments. Internal details of the temperature profile and oxygen behavior are not predicted as well. These discrepancies

have suggested certain model refinements, in particular, a variable area treatment of the coal region and addition of inward oxygen diffusion through the cigarette paper. These changes have been included in the computer program and are being studied parametrically. When this is completed, the next step is addition of adsorbed water behavior as a prelude to further refinements in the chemistry of the tobacco and the thermophysics of the smoking cycle, as indicated above. By the end of the fiscal year, the thermophysics of the model should describe a realistic puff/relaxation cycle.

Significance to Biomedical Research and the Program of the Institute: The experimental development of less hazardous cigarettes depends on the identification of variables that cause adverse health effects and on the manipulation of the variables to diminish those effects. A substantial experimental program is being carried out to accomplish this. Variables have been selected on the basis of experiment and judgment and have been manipulated singly or in combination. Tumorigenesis and other adverse health effects of the resulting experimental cigarettes have been measured, and the results have been combined with other inputs to design further experiments. Each experiment is a large and extensive effort, lasting for 18 months and capable of handling some 25 to 35 experimental cigarette types. With a complex and extensive field to explore, the NCI needs a method to select the most important cigarette design parameters; in this way, the logistically demanding large experiments will evaluate only the most promising new designs of potentially less hazardous cigarettes.

There is reason to believe that computer simulation may assist in screening alternative approaches to the design of less hazardous cigarettes. The concept, in brief, is a model based on established relationships in physical and chemical science, with input variables that are controllable in the makeup of cigarettes and outputs describing the quantity and physical state of any particular smoke component for each selected set of input variables.

Proposed Course: The contractor will undertake computer simulation efforts according to the phase plan briefly described above. Periodic reviews of the model and comparison with experimental data will suggest continuing refinements to assure simulation fidelity.

Date Contract Initiated: March 1, 1976

Current Annual Level: $85,000

ROSWELL PARK MEMORIAL INSTITUTE (SHP-75-113)

Title: Bioassay of Cigarette Smoke Condensate by the Sebaceous Gland Suppression Test

Contractor's Project Director: Dr. Fred G. Bock

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: To determine the sebaceous gland suppression (SGS) index of experimental cigarette smoke condensates that have been or are being tested for carcinogenic activity in mouse skin. Multiple regression equations relating the SGS index, chemical analytical data, and carcinogenic potency may then be used to select the most promising candidate cigarettes for long-term studies.

Major Findings: The SGS indices for the experimental cigarettes have been determined. Preliminary comparison of these findings with the carcinogenic activity of the cigarettes from Series I to III shows good correlation ($r=.83$) between the SGS index and carcinogenic potency. The SGS index is correlated with nicotine content ($r=.56$) but not with benzo[a]pyrene.

Significance to Biomedical Research and the Program of the Institute: The SGS index provides another criterion by which promising candidate cigarettes can be selected by short-term assays. The study will be of further value if the sebaceous gland suppressor of smoke condensates is subsequently identified. If so, we would have a better selection of chemical indicators of the carcinogenesis potential of cigarettes.

Proposed Course: Within the next 60 days, the carcinogenic activity and chemical composition of the Series IV cigarettes should be available. At that time, the correlation of SGS index with several of these parameters will be completed.

Date Contract Initiated: February 16, 1976

Current Annual Level: $8,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (SHP-74-102-SA1)

Title: Inhalation Testing of Cigarette Smoke in Baboons

Contractor's Project Director: Dr. Walter R. Rogers

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: This project aims to 1) demonstrate the ability of the baboon to serve as a surrogate cigarette smoker similar to the human; 2) collect behavioral, physical, and chemical dosimetry data as the animals smoke different types of cigarettes in controlled experiments; 3) develop pulmonary lavage methods to verify deposition of particulate matter in the lower respiratory tract; 4) conduct experiments to detect possible nicotine dependence in smoking animals; 5) develop an automated cigarette dispenser/ejector system; and 6) make recommendations as to the feasibility of using the baboon as a model to study the effects of cigarette smoking, especially on the pulmonary and cardiovascular systems.

Major Findings: To date the project has 1) successfully developed the instrumentation and training techniques needed to conduct controlled experiments with the cigarette-smoking baboon; 2) trained 12 baboons to smoke

cigarettes in a human-like fashion, making 400 to 500 puffs on 48 cigarettes in a 12-hour period; 3) demonstrated that smoking behavior can be maintained and controlled for periods as long as 3 years; 4) found that cigarette smoking baboons develop blood levels of carboxyhemoglobin, nicotine, cotinine, and thiocyanate similar to most human cigarette smokers; 5) measured smoking puff profiles and found that the puff volumes, durations, and pressures are essentially the same as those of human smokers, and 6) demonstrated that significant deposition of particulate matter occurs in the lung as the animals smoke cigarettes.

Significance to Biomedical Research and the Program of the Institute: This project has clearly demonstrated that the baboon can be used as a nonhuman primate model of cigarette smoke inhalation to test selected cigarettes for their immediate and long-term effects. The primate model, which provides many unique advantages, can be used for such research at costs that are competitive with the costs of other animal models currently used for this purpose.

Proposed Course: Once the current project is completed, the next step will be to exploit the cigarette-smoking baboon model to identify substances in cigarettes that should be modified or eliminated and to examine how alterations in cigarette composition and manufacture can reduce the health hazards associated with smoking.

Date Contract Initiated: October 1, 1975

Current Annual Level: $103,000 (July 1, 1976 - August 31, 1977)

U.S. DEPARTMENT OF AGRICULTURE (YO1-CP6-0209)

Title: Modification of Tobacco Through Production and Curing Process

Contractor's Project Director: Dr. T. C. Tso

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The chemical composition of cigarette smoke depends on the characteristics of leaf tobacco used for the manufacture of the cigarettes. Production and curing practices, in turn, determine the characteristics of tobacco leaf. Modification of tobacco through the production and curing process may therefore change the leaf as well as the cigarette smoke. These projects involve various production, curing, and postharvest handling variables, followed by smoke chemistry and biological evaluation for the purpose of developing a less hazardous cigarette.

Major Findings: ARS is responsible for coordination with the North Carolina State University; the Oxford, N.C. Tobacco Station, ARS; AMF; Peter Schweitzer, Inc.; the cigarette industry; AHF; Orchard Park Laboratory; and New York Medical College for six different projects: 1) production of tobacco by high density and normal density, involving the use of the whole plant including stalks or leaves only; 2) use of two curing methods, conventional curing of

"chopped" plant material vs. homogenized leaf curing followed by two methods of reconstitution; 3) production of "deproteinized" tobacco for the purpose of reducing HCN and other undesirable nitrogenous compounds; 4) postharvest treatment for reduction or removal of radioelements or soluble proteins from cured leaf; 5) production of leaf tobacco enriched with Pb-210 followed by smoke and bioassay; and 6) in view of possible use of stalk and other fibrous materials for sheet manufacture and cigarette smoke, production of C^{-14} labeled cellulose and other fibrous materials for sheet reconstitution and smoke characterization.

In 1975 and 1976, tobacco materials were produced in Projects 1, 2, and 4, and smoke chemistry and bioassay evaluating these materials are in progress. Project 3 will be conducted during the 1977 crop season at Oxford, N.C. Project 5 is in progress for Pb-210 cigarette manufacture and animal inhalation, followed by histopathology in 1977-1978. Project 6 will be conducted during the year 1977-1978.

Significance to Biomedical Research and the Program of the Institute: These six projects will take another year to complete. Results obtained will provide basic information on how to reduce undesirable smoke components through production, curing, and postharvest handling. For example, we expect to study the role of radioelements in health, the effect of cellulose and fibrous materials used for sheet reconstitution and smoke composition, and the effect of proteins in relation to HCN and other nitrogens. Knowledge gained from these studies will be invaluable for developing a less hazardous cigarette.

Proposed Course: Materials produced under projects in 1976 are being used for smoke chemistry study and skin painting tests. Leaf tobacco containing radioelements will be made into cigarettes to be used for animal inhalation studies in 1977. Deproteinized tobacco will be produced in 1977. We expect that most of the tests will be completed by the end of 1978 or early 1979. Most of these projects are supported jointly by NCI and ARS, for the mutual objective of producing a safer tobacco and a less hazardous cigarette.

Date Interagency Cooperative Agreement Initiated: February 9, 1976

Current Annual Level: $57,000

VETERANS ADMINISTRATION HOSPITAL, EAST ORANGE, NEW JERSEY (YO1-CP4-0205)

Title: Effects of High- and Low-Nicotine Cigarettes on Male Beagle Dogs

Contractor's Project Director: Dr. Oscar Auerbach

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: 1) To evaluate the role played by nicotine in the development of pulmonary and cardiovascular disease associated with chronic smoking of cigarettes. 2) To determine whether nicotine is importantly involved in the harmful effects produced in male beagle dogs by daily inhalation of

cigarette smoke. 3) To determine the significance of the development of pulmonary and cardiovascular diseases,which include proliferative and dysplastic changes in the tracheobronchial epithelium, pulmonary interstitial fibrosis and emphysema, arteriosclerosis, and bronchiolar epithelial changes. 4) To determine the significance of the development of pulmonary and cardiovascular disease during chronic smoking, using male beagle dogs as described in the FY 1977 proposal for renewal.

Major Findings: The study concerns itself with the respiratory exposure of male beagle dogs to smoke from cigarettes of high- and low-nicotine content. The smoke is administered via tracheostoma from smoking machines designed by Arthur D. Little, Inc. (ADL), of Cambridge, Mass.

The ADL machine delivers a puff volume of 35 cc of smoke at 20-second intervals, providing three puffs per minute per dog. The animals receive six cigarettes in the morning and six cigarettes in the afternoon, 7 days a week.

Thus far in the study, both high and low nicotine smoking dogs have died and have been extensively examined. The findings definitely indicate that the dogs are receiving a good dose of cigarette smoke and that there are definite changes related to cigarette smoking. There are changes within the lung tissue which indicate the damage done by the inhalation of cigarette smoke. The following are summaries of the findings in these dogs.

Dog AGM-20 of the high-nicotine group succumbed on June 29, 1974. The animal had smoked 1,004 high-nicotine cigarettes in 170 smoking days. Multiple infarcts were present in six of the seven lobes of the lung. The dog died of pulmonary insufficiency. Bronchopneumonia was present, and abscesses were found in the right intermediate and right diaphragmatic lobes of the lung. There was evidence of a pulmonary fibrosis and emphysema, being more advanced in the upper lobes. A point of interest was the presence of basal cell hyperplasia within the bronchial epithelium and beginning atypism in basal cells of all parts of the tracheobronchial tree.

A second animal (MB-30) of the high-nicotine group succumbed on October 5, 1974. The dog smoked a total of 2,192 high-nicotine cigarettes in 269 smoking days. The essential findings were those of a pulmonary fibrosis and emphysema of a slight to moderate degree, present chiefly in the subpleural aspects of the lobe. In addition to the fibrosis and emphysema, there was also a basal cell hyperplasia of the bronchial epithelium of a slight to moderate degree with slight atypism. This was seen in the atypical nuclei present in the basal layers of the bronchi. The dog also developed a thrombosis in the right and left auricular appendages with resultant emboli and pulmonary infarcts in the left apical, left diaphragmatic, and right intermediate lobes. The changes in this dog are not much further advanced than those present in AGM-20, who died after 170 days of smoking.

Dog JV-82 of the high-nicotine group died during the morning smoking session on December 16, 1974. He had smoked 3,048 high-nicotine cigarettes in 341 smoking days. In this dog, the findings were not as extensive as one would

have expected when compared with the findings in dog AGM-20. There were only slight pulmonary fibrosis and emphysema in the lungs. The changes in the lungs also showed the presence of fat and brown pigment granuloma, which were the result of the inhalation of the particles in the cigarette smoke. The lumen of the right ventricle was greatly dilated. Usually when dogs die while they are smoking, the cause of death in the majority of instances is that of an extensive right heart failure. Here the right ventricle was extensively dilated and the apex was rounded.

Additional points of interest in this case were the foci of hemorrhage into the alveolar spaces and also the occasional small focus of organized bronchopneumonia and obliterative bronchitis.

Dog NC-01 of the low-nicotine group succumbed on March 10, 1975. The animal had smoked a total of 4,058 low-nicotine cigarettes in 425 smoking days. He died while being treated for an infected cyst located between two toes. The animal struggled constantly during treatment and required restraint. The cysts were drained and injected with penicillin G and dihydrostreptomycin. The animal went into a convulsive seizure under restraint and became comatose. Artificial respiration was administered for 10 to 15 minutes but to no avail. The autopsy findings were those of bronchiolar proliferation. The bronchi and surrounding glands were lined by cuboidal epithelial cells similar to those seen in bronchiolo-alveolar tumors. The other findings were a pulmonary fibrosis and emphysema of a slight to moderate degree, more advanced in the upper lobes. In addition, there was slight thickening of the pulmonary arterioles. There was a basal cell hyperplasia. The only difference between this and the heavy smoking dogs is that there was a little more atypism in the basal cells of the heavy smoking dogs than in this group. However, the changes of pulmonary fibrosis and emphysema were present.

Interesting in this case was the presence of such a large number of fat and brown pigment granuloma.

Dog MG-32 of the high-nicotine group expired on May 4, 1975. He had smoked a total of 4,718 high-nicotine cigarettes for 480 days. On May 2, 1975, the animal was given 1.5 cc of "Inovar Vet" (analgesic tranquilizer). Tartar was removed from the teeth, and the ears were cleaned. The animal recovered from the tranquilizer by 4 p.m. and was up and about in its cage. On May 3, 1975, the animal was smoked by weekend personnel. There was no report of any adverse effects.

There were thrombi in the left and right auricular appendages, not an infrequent finding in dogs who have smoked in the high-nicotine group. There were emboli to the left apical and left diaphragmatic lobes. The dog had a rather extensive bronchopneumonia, present in all lobes of the lung. There was also an acute suppurative bronchitis that had resulted in a great denudation of the bronchial epithelium throughout its entire length. Where the epithelium was intact, basal cell hyperplasia was present and was of a moderate degree. The epithelium of the trachea was squamous in character and showed a moderate atypism with some rather large nuclei present in these zones. There was evidence of pulmonary fibrosis and emphysema, more advanced

in both apical lobes. There were granulomas within the lung and lung worms in the center of some of these granulomas. Another interesting feature was the presence of a cylindrical bronchiectasis within the right intermediate lobe. The lumens of the bronchi were filled with inspissated material with a ridging of the bronchi. In conclusion, there was evidence of basal cell hyperplasia with atypism, pulmonary fibrosis and empnysema,and lung worms in the lung.

Dog AW-02 of the low-nicotine group succumbed on November 5, 1975. He had smoked a total of 6,194 low-nicotine cigarettes up until the time of his death. He had smoked for 663 days. The animal was pulled from the smoking line before he finished the last cigarette. It was observed that the smoke was not going down into the trachea, but instead was going out under the animal's skin with subcutaneous emphysema of an advanced degree. Needles were applied to the skin in an attempt to release the trapped air. The animal was given 10 mg of promethazine hydrochloride in an attempt to counteract the edematous tissue swelling. There was an attempt to keep the air passage open. Before the animal died, artificial respiration was applied and also an emergency enlargement of the tracheostomy opening was accomplished. An autopsy performed immediately showed the tracheostomy opening to be somewhat blocked. The outstanding feature in this case was the minimum amount of pulmonary fibrosis and emphysema, which was present in the lungs, where it occurs chiefly in the left apical and right apical lobes. The arterioles within the lungs were not thickened. It is noteworthy that, with the exception of the slight thickness of the arterioles in the periadrenal tissue, there was no evidence of arteriolar thickening in the body. Within the bronchi and within the trachea, the signs of atypism in the basal nuclei were slight. The basal cell hyperplasia within the bronchial glands was of a slight degree.

Another animal (VX-40) of the high-nicotine group expired on January 10, 1976. He had smoked 7,700 cigarettes in 718 smoking days. The interesting findings are those of extensive actinomycotic abscesses in the mediastinal cavity and also in the left apical lobe. One gains the impression that the lesions began in the left apical lobe and extended to the pleura. The slides on Dog VS-40 were submitted to Dr. Svend Nielsen, Department of Pathobiology at the University of Connecticut in Storrs, Conn., who has written on the subject of canine actinomycosis. He believes that the case is one of actinomycosis with an additional complication of gram-positive organisms. Microscopically, the lesions in the lung were those of a granulomatous process with the actinomycosis present in some of the abscess cavities. There was filiform granulation tissue in the pleura. Extensive pulmonary fibrosis and emphysema were not found, except in scattered sections of the lung parenchyma. This may be accounted for by the fact that the lungs were compressed by the extensive granulomatous process present both in the mediastinum and in the pleural cavities. Of interest is the fact that there were several granulomas in the lung. Although the worms were not present in their center, they did appear very much like the lung worms that we have observed in the previous dogs in this study. The other findings in this case were slight arteriolosclerosis in the pulmonary arterioles and also slight pulmonary arteriolosclerosis in the coronary blood vessels. Fat and

brown pigment granulomas were present both in the lung and also in the tracheobronchial tree. There were also small infarcts in the left diaphragmatic as well as the right diaphragmatic lobes. This is the first instance in all dogs that we have studied in which actinomycosis was observed.

Dog YB-40 of the high-nicotine group expired on June 26, 1976. The animal smoked 9,662 cigarettes in 892 smoking days. At the time of the autopsy, the outstanding feature was the presence of an extremely advanced cor pulmonale with great dilation of the right ventricle so that the papillary muscles and trabeculae corneal appeared flattened, with the apex of the right ventricle extremely rounded. In addition, there was evidence of a bronchopneumonia of recent duration in five of the seven lobes. These were of varying degrees. There was also an acute bronchitis present in four lobes. A point of interest in this case is that, in spite of the fact that the dog had smoked 892 days with a high-nicotine-type cigarette, the changes of pulmonary fibrosis and emphysema were not as severe as those that we have seen in several of our previous studies. Also, there was a lack of squamous metaplasia of the tracheobronchial epithelium and a lack of real atypism of the bronchioles. A point of interest is the extent of atypical changes in the wall of the urinary bladder.

Dog AGZ-30 was found dead in his cage on August 1, 1976. He was of the low-nicotine group and had smoked 10,208 cigarettes in 937 smoking days. On July 29, 1976 the dog was vomiting and had a bloody diarrhea. His temperature was 102^{2}°F. The lungs were congested, and the animal was weak. The animal was treated with antibiotics and intestinal medication. The dog was not smoked from the onset of his illness until his death.

The essential findings in this case were, as far as smoking is concerned, a pulmonary fibrosis and emphysema of a slight to moderate degree, more advanced in the left apical and right apical lobes. In addition, there was a basal cell hyperplasia of the bronchial epithelium. This was of a slight to moderate degree, but the atypism was slight. The dog died of an extensive bronchopneumonia that was present in five of the seven lobes, sparing the left diaphragmatic and right diaphragmatic lobes. In addition, there was evidence of a focal scar in the left diaphragmatic lobe, which may have been due to a healed inflammatory process. We do not know the origin, because this scar was beyond recognition of any underlying process. An incidental finding was the focal calcification in the right apical lobe. Several other interesting features were present in this case. There was a slight thickening of the coronary arterioles as well as a slight thickening of the pulmonary arterioles. Preceding this case was the death of high-nicotine dog YB-40, who had smoked for 892 days and had smoked 9,662 cigarettes. This case and YB-40 were presented to the Tobacco Working Group at a meeting on Wednesday, September 8, 1976, in Bethesda, Md. In presenting the cases to the conference, it was pointed out that the differences between the high-nicotine and low-nicotine dogs, in the opinion of the observers, were not as great as one would have expected. At this meeting slides were also shown of the old smoking study using the Kirman machine, in which the changes were much more advanced than in YB-40 or AGZ-30. Since AGZ-30 died, there have been two other dogs who have died after smoking low-nicotine cigarettes.

Dog AAV-42 of the low-nicotine group was found dead in his cage on August 5, 1976 after an illness of several days. On August 3, 1976 he did not eat. His temperature at that time was 103°F. His lung sounds were compatible with a severe pneumonia. He was given antibiotics and he became weak and near death on August 4, 1976. He had smoked 10,274 cigarettes during a period of 943 days. The autopsy findings, as far as the lungs were concerned, showed a slight to moderate pulmonary fibrosis and emphysema. In addition, verifying the clinical impression was the presence of both an acute bronchitis and a severe bronchopneumonia in all lobes of the lung. It was difficult to evaluate completely the changes in these lungs, because the dog was found dead and had a severe bronchopneumonia. Of extreme interest in this case was the presence of amyloidosis within the liver, spleen, kidneys, adrenals, pancreas, and probably in the blood vessels throughout many of the organs. It is frequently observed in cases of pulmonary tuberculosis, less frequently in chronic infections. The distribution of the amyloid within the liver, spleen, kidney, and adrenals is the type that is seen in secondary amyloidosis. The autopsy in this case did not show any evidence of chronic infection. Amyloidosis has been recorded in dogs. In our studies, we have seen several dogs with severe amyloidosis who have died of an amyloid uremia. The reason for our believing that this is an amyloid uremia is that, in studies on human beings, we found that, when the glomeruli are completely occupied by amyloid deposition and Bowman's space obliterated, the clinical picture was always that of amyloid uremia. What is puzzling in this case is that there is no obvious reason for the amyloidosis developing in these organs. It certainly cannot be considered as a primary amyloidosis, because the location is not primarily within the heart, esophagus, or bladder sites.

Dog BZ-01 of the low-nicotine group was found dead in his cage on the morning of September 7, 1976. He had received Dilantin on a regular basis, but showed no evidence of having died in a convulsion. The animal had smoked 10,183 cigarettes for 947 smoking days. The reason for the low total of smoked cigarettes is the fact that, when the dog had convulsions, he was removed from the smoking machine and not smoked for a period of days. The anatomic findings were those of an extensive bronchopneumonia that involved all lobes of the lung. The process was more advanced in the apical than in the diaphragmatic lobes. In addition, there were a pulmonary fibrosis and emphysema of a slight degree. This was less than the two previous dogs that died following smoke inhalation of low-nicotine cigarettes. In addition there were fat and brown pigment granulomas in the lungs. The pulmonary arterioles, the coronary arterioles, and the arterioles in the periadrenal regions were slightly thickened. The basal cell hyperplasia present in the tracheobronchial tree was slight in amount and showed only slight atypism.

The main changes present in this case were the slight basal cell hyperplasia with slight atypism, as well as the slight pulmonary fibrosis and emphysema in the lobes of the lung, which are compatible with low-nicotine cigarettes. Another dog dies 1 month later. This dog was in the high-nicotine group. The cause of death in this case was advanced bronchopneumonia that involved all lobes of the lung.

Dog GC-52 of the high-nicotine group had smoked 10,946 cigarettes during a period of 999 days. On the day of the animal's death he had been placed in

the smoking box at 8:00 a.m. The dog was not smoking, but saliva was being passed in anticipation of the smoking. During this time, it was noticed that the dog had become weak, and artificial respiration was administered immediately but to no avail. He died on October 6, 1976.

There are several interesting features in this case. This is the first dog in this series in which we have observed a bronchioloalveolar tumor. This is the type of tumor that has been described in literature in human beings and fits all the criteria described by those who have studied this type of tumor. The changes of the bronchioloalveolar tumor in this case are those of a central bronchiole surrounded by small glands. The lining of the bronchioles, as well as of the glands in the surrounding areas, is composed of cuboidal and columnar cells. The lumens of the bronchiole communicate with some of the surrounding glandular structures (Labert's Canals).

A feature that is present in dogs and not seen as frequently in human beings is the presence of a squamous metaplasia in some of the glands of the bronchioloalveolar tumor. Liebow in his discussion of the bronchioloalveolar tumors states that squamous metaplasia occurs only in about 25% of the bronchioloalveolar tumors in human beings. In dogs we have seen this as a constant finding. In a previous series, we found squamous metaplasia with extensive atypism almost exclusively in the nonfiltered cigarette smoking dogs.

The other finding in this case was that of many granulomata that are composed of brown pigment, as well as fat and histiocytes. In addition, many of these foci showed an abundance of collagen fibrils. Surrounding these was a dilatation of the alveolar spaces (perifocal emphysema). The changes in the lung parenchyma were those of a pulmonary fibrosis and emphysema of a slight to moderate degree. The changes, however, were not as extensive as those that we have observed in a previous study and were not much greater than those that we have observed in the low-nicotine smoking dogs in this series. Of interest in this case is the advanced cor pulmonale. It has been our impression thatthe cor pulmonale occurrred while the dog was smoking. It is of interest that, in this case, the dog was not smoking at the time of his death; in spite of this, he died of cor pulmonale. This is a rather unusual occurrence in all of our series. Another point of interest in this case is the presence of Type II cell proliferation. This observation was made in a previous series of smoking dogs. Here again, the changes were present in the dogs who smoked for a long period of time and then only in those dogs who were in the unfiltered cigarette smoking group. Again it will be of interest to see whether these changes become more pronounced as the dogs continue to smoke high-nicotine cigarettes. Thus, in this case, the presence of the bronchioloalveolar tumor and the Type II cell proliferation differentiated it from the low-nicotine cigarette smokers. The other changes in this case were those of a rather intense passive congestion and the basal cell hyperplasia of the trachea and the bronchi of a slight to moderate degree.

Dog QE-01 of the low-nicotine group was found dead in his cage on November 11, 1976 after an illness of several days duration. Upper respiratory infection was present and was treated with antibiotics. The animal carried one degree

of fever, but was not able to throw off the infection. The animal had smoked 11,396 cigarettes in 1,036 smoking days.

The interesting findings in this case are those of an infiltration in all lobes of the lung. It was present in the trachea and bronchi, as well as the lung parenchyma. In addition, similar changes were found in the spleen, the testes, the wall of the colon, and the cervical lymph nodes. The cells appeared to be those of mastocytes. These slides will be taken to Dr. Svend Nielsen in Storrs, Conn. Dr. Nielsen is the individual who has done so much work on this lesion. The changes in the lung were of a slight to moderate basal cell hyperplasia with atypism, as well as a slight to moderate pulmonary fibrosis and emphysema. The overwhelming lesion at the time of death was a diffuse bronchopneumonia from which the dog died. The question of the mastocytosis will be answered. The coronary arterioles showed a slight to moderate thickening but not as extensive as would be expected at the period of 1,036 days.

Hematological parameters and clinical chemistry values being measured in this study include the total red blood cell count, total white blood cell count, the differential white blood cell count, hematocrit, and hemoglobin determinations. Platelet aggregation, blood cholesterol, and carboxyhemoglobin values have also been measured. It is hoped that in the future nicotine and cotinine values can also be determined.

Due to the fact that limited blood samples have been collected to date in the study, it is too early to evaluate the results. No appreciative difference has been seen between control animals and test animals, except that white cell counts appear to be higher in the test animals, generally associated with an increase in the relative percentage of polymorphonuclear leukocytes. This would seem to indicate a low-grade infection in test animals.

Cholesterol levels were generally higher in test animals than in the controls, with tremendous individual variations.

Initially in our platelet aggregation tests it was clear that the platelets of the test animals aggregated much more easily with a lower concentration of aggregating agent than did the platelets of control animals. This would have implications in terms of tendency to thrombosis. However, this effect was not seen in later tests. Presently numbers are too small to evaluate.

Carboxyhemoglobin measurements show that the basal carboxyhemoglobin levels are about 3% in smoking or nonsmoking dogs. Smoking boosts the carboxyhemoglobin about 3% for each cigarette smoked when the measurement is taken soon after smoking. We have no information on the rate of disappearance of the carboxyhemoglobin.

Our blood analysis work is being carried out by the College of Medicine and Dentistry of New Jersey under the capable hands of Jack Weinstein, M.D., Director of the Hematology Department.

A collaborative study is ongoing with the Senior Medical Investigator of the Bronx, New York Veterans Administration Hospital, regarding ACTH production in smoking beagle dogs. Plasma samples of our smoking beagle dogs on a 2-month interval are being utilized in the study, as well as appropriate tissue samples on postmortem. The study is to determine whether plasma sampling is of value in predicting whether smoking dogs develop pathological changes associated with ACTH production and whether there is a correlation between histological changes and ACTH production in lungs of smoking dogs taken on postmortem. Results of the study are still pending.

An additional study is planned, in which the scanning electron microscope will be used to detect the earliest morphologic changes produced in the distal respiratory system of smoking dogs, as outlined under New Experimental Procedures in the FY 1977 Proposal for Renewal. In this study, 12 additional dogs will be utilized and will be on high-tar, high-nicotine cigarettes. The latex injection technique of Wang proved to be very difficult to handle due to its extreme fragility. A new latex experimental procedure is being employed on guinea pigs before proceeding with the experimental dogs. This new technique has proved, thus far, satisfactory on the few guinea pigs tried. We intend to do several more guinea pigs before proceeding to the experimental dogs. The scanning electron microscope was not used in our previous microscopic evaluations of smoking dogs.

An adjunct study is also being proposed to elucidate further the effects of cigarette smoking on the immune system in dogs, according to the unique experimental design that is ongoing in our present study of the Effects of High- and Low-Nicotine Cigarettes on Male Beagle Dogs. Recent reports have linked increased susceptibility to respiratory infection to cigarette smoking. There is considerable evidence to suggest that cigarette smoke and products derived from cigarette smoke have immunosuppressive properties.

Bioassay monitoring of our long-term dog smoking study is still being accomplished by the Bio/Organic Analysis Group of the Tobacco Research Program, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Serum samples continue to be collected from our smoking dogs on a periodic basis and are being held for long-term studies (a serum bank) by the NCI.

Significance to Biomedical Research and the Program of the Institute: It is believed that the developing pathology of the tissues of the experimental animals in this study will provide some clues to the role that nicotine plays in possibly being a major contributor to the harmful effects of cigarette smoking.

Results from autopsy studies may show the changes in the bronchial mucosa that are thought to precede development of frank bronchogenic carcinoma. Is there a difference between high- and low-nicotine doses of chronically inhaled smoke in male beagle dogs in the development of hyperplasia, metaplasia, or the process called metastasis?

It is hoped that this research will provide new data on the interrelationship of cardiopulmonary and carcinogenic effects of cigarette smoke and nicotine.

Date Interagency Cooperative Agreement Initiated: April 1, 1972

Current Annual Level: $359,000

VETERANS ADMINISTRATION HOSPITAL, MIAMI, FLORIDA (YO1-CP6-0207)

Title: The Detection of Early Obstruction of Respiratory Airways

Contractor's Project Director: Dr. George L. Baum

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: 1) To determine whether any abnormal findings are related to any type of cigarettes previously smoked. 2) To detect any evidence of early lung disease, even though there are no symptoms relating to the lungs and the chest X-ray is normal.

Major Findings: Data collection has been completed. A total of 487 subjects were entered into the study. Preliminary analysis comparing asymptomatic subjects who smoked a total of 5 or more pack-years of cigarettes with a control gorup of asymptomatic nonsmokers indicates the following.

1) No significant difference between groups is found for conventional spirometry and lung volumes, airway conductance, single breath, or diffusing capacity.

2) Maximum expiratory flow rates at low lung volumes have a large variance within the control groups. This finding makes the test less useful as a screening device than (3) and (4).

3) The single breath nitrogen test has two components useful for screening: Phase IV (closing volume)/VC ratio and slope of the alveolar plateau. Of 190 smokers, 22 had abnormal Phase IV/VC and 31 had abnormal slopes. Only seven subjects had both abnormal Phase IV/VC and abnormal slopes.

4) A new index was established for analysis of a multibreath nitrogen washout test. Of 200 smokers, 33 had an abnormal index, 17 of whom had an abnormal slope on the single-breath test, and only 6 had abnormal Phase IV/VC.

5) A correlation between number of pack-years of smoking and increased incidence of abnormal results was demonstrated.

Significance to Biomedical Research and the Program of the Institute: This project is important because its goal is to determine an optimal screening test for early airways obstruction.

Proposed Course: Further analysis of data is in progress, and publication of results is planned.

Date Interagency Cooperative Agreement Initiated: June 1, 1976

Current Annual Level: $20,000

Projected Programs

The plans for the future include expanding inhalation bioassays to determine the physiological effects of chronic exposure to nicotine, carbon monoxide, NO_x, and HCN in cigarette smoke; developing techniques for measuring human dosimetry in cigarette smoking; continuing investigations of the relationship between smoking habits and tobacco-related diseases; extending epidemiological studies to assess the health effects of reduced hazard cigarettes; and cooperating in the development of risk analysis techniques.

Future tasks of the Smoking and Health Program are as follows:

- To clarify chronic nicotine toxicity.
- To develop methods for further reduction of carbon monoxide.
- To develop methods of reducing cigarette smoke inhalability.
- To develop methods of identifying susceptible individuals.
- To help persuade industry and consumers to adopt less hazardous cigarettes.
- To expand epidemiological surveys of possible preventive results.

Various research areas are discussed below.

Experimental Cigarette Series. The results of the mouse dermal assays of the first, second, and third series of experimental cigarettes have been evaluated. Fourth series skin painting is in progress, and the fifth series is being designed. New technologies incorporating reconstituted tobacco sheet and tobacco extenders will be utilized in this series, and consideration will be given to how particular constituents can be eliminated by cigarette construction or by smoke filtration. At this time, only chemical analysis and inhalation bioassays are being planned for the fifth series of cigarettes.

Chemistry. In the future, analytical chemistry will still be necessary to characterize the composition of the smoke from candidate less hazardous cigarettes used in inhalation bioassays. However, new techniques, such as chromatographic profiling, will also be applied. Increased attention will be paid to the physical-chemical mechanisms involved in the formation of particular chemical constituents of cigarette smoke during pyrolysis.

Bioassay Projects. Table 6 lists the bioassay projects that are expected to be active in the immediate future. Of the 14 projects, 9 are inhalation

studies concerned with assaying exposures under conditions similar to those experienced by human smokers.

Flavor and Acceptability. To the greatest extent possible, work will be accelerated on the development of desirable organoleptic and acceptability characteristics that could be added to stripped or extended tobaccos without reintroducing toxicity or long-term health hazards. Candidate flavor constituents will be assayed singly and in combination, both as additives and as components of cigarette smoke. Furthermore, the Smoking and Health Program will be receptive to new approaches to the analysis and construction of cigarette flavor.

In addition, continued attention will be paid to understanding the role of nicotine, as well as social and psychological factors, in acceptability and habituation.

Dosimetry. To make complete use of the research information now available, it will be necessary to evaluate human smoking patterns accurately. Efforts will be directed towards the development of equipment and techniques for measuring the dose and deposition of cigarette smoke in human subjects. Results of the large-scale application of these techniques will aid in interpreting risk levels associated with smoking. In addition, these data on dose and deposition will be used in designing future inhalation bioassays.

Potential for Reduction of Smoking-Related Diseases

It is difficult to discern trends in the incidence of smoking-related diseases. Extrapolating incidence figures is possible when the trends are clear, but such extrapolations must assume that there are no substantial changes occurring. Changes are being made, however, and there is some evidence to suggest that these changes are reducing the incidence of some smoking-related diseases.

Retrospective and prospective studies showing a dose-response relationship indicate that a reduction in exposure to tobacco smoke should result in reduced risk of smoking-related diseases. During the past 20 years, a dramatic reduction in the tar and nicotine yields of American commercial cigarettes has taken place (Table 7). Recent epidemiological studies suggest that, to develop a certain risk of disease, a person would have to smoke more of today's cigarettes than of those available 20 years ago. These studies also indicate that a person who has smoked filter cigarettes for 10 years or more has a significantly lower risk of lung cancer than a person who has smoked unfiltered cigarettes for the same period. Recent data on mortality from lung cancer in the United States indicate that rates are declining for most age cohorts in the male population and that some negative slopes have actually appeared in the younger cohorts. Figure 7 depicts the 5-year slope change in death rates from malignant neoplasms of the trachea, bronchus, and lung.

Results obtained from Smoking and Health Program activities indicate that the hazards of cigarettes can be decreased by reducing tar, eliminating

Table 6

Ongoing and Anticipated Bioassays

Investigator	Bioassay	Application/Purpose
Arthur D. Little, Inc.	Ciliatoxicity	Series III & IV
	Alveolar Macrophage Inhibition	Series III & IV
	Cytotoxicity	Series III & IV
Hazleton Laboratories America, Inc.	Dog Inhalation	Effect of Cigarette Smoke on Pulmonary Function
	Dog Inhalation	Effects of Nicotine, CO, NO_x on Atherosclerosis
Veterans Administration Hospital, East Orange, N.J.	Dog Inhalation	Bioassay Demonstration High and Low Nicotine
Battelle Pacific Northwest Laboratories	Dog Inhalation	Bioassay Evaluation Role of Nicotine
	Rat Inhalation	Bioassay Demonstration
Oak Ridge National Laboratory	Rat Inhalation	Bioassay Development
Southwest Foundation for Research and Education	Baboon Inhalation	Feasibility Study
Arthur D. Little, Inc.	Bronchial Epithelium Implantation	Feasibility Study
Roswell Park Memorial Institute	Sebaceous Gland Suppression	Bioassay Evaluation Prescreen Development
Harvard University	Pigeon Inhalation	Atherosclerosis and Smoking
To Be Announced	Dog Inhalation	Flavor-Enhanced Cigarettes

Table 7

Trends in Tar and Nicotine Levels

Year	Average Yield (mg)/Cigarette	
	Tar	Nicotine
1955	43	2.8
1967	21.78	1.37
1968	20.92	1.39
1969	20.73	1.38
1970	20.32	1.33
1971	20.12	1.32
1972	19.82	1.37
1973	19.24	1.32
1974	18.74	1.27
1975	18	1.2

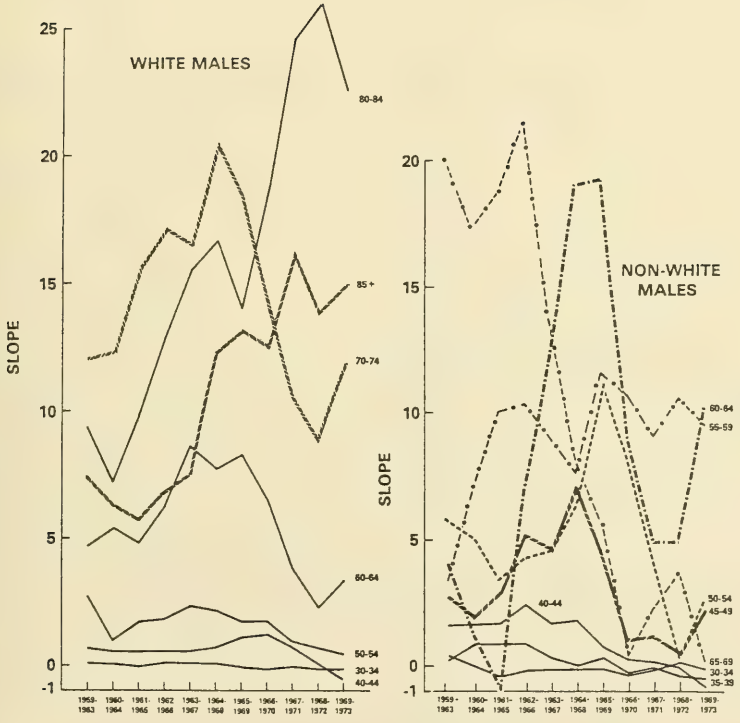


Figure 7. Five-year changes in death rates from malignant neoplasms of the trachea, bronchus, and lung.

toxic smoke components, and adjusting nicotine levels to achieve consumer acceptance. The necessary technology has already been established. There is also evidence that consumers will accept the "new cigarettes"; this is reflected in the current sales of cigarettes with lower levels of tar and nicotine than those marketed 15 years ago. Future commercial cigarettes can be expected to utilize compensating flavors and fragrances, while still maintaining less hazardous characteristics.

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Annual Report
Diet, Nutrition and Cancer Program
Division of Cancer Cause and Prevention
National Cancer Institute

July 1, 1976 through September 30, 1977

Dr. Gio B. Gori, Director

INTRODUCTION

In response to a mandate in the 1974 amendments to the National Cancer Act, the National Cancer Program developed the Diet, Nutrition and Cancer Program. The purpose of the program is to develop and disseminate information on the interrelationships between diet and nutrition in the etiology of cancer and the therapy and subsequent rehabilitation of the cancer patient. Administratively the program is managed by the National Cancer Institute, Division of Cancer Cause and Prevention (Figure 1). Overall policy and direction are provided by an Advisory Committee composed of scientists from government, academia, and industry. Specific research projects to be supported by the program are formulated by researchers in program definition workshops. These researchers represent various scientific disciplines such as epidemiology, clinical oncology, human nutrition, anthropology, biochemical metabolism, animal nutrition, and the behavioral sciences. The projects formulated by the workshops are ranked by the Advisory Committee and executed by both the contract and grant mechanisms.

BACKGROUND

It is apparent that many chronic diseases such as heart disease, cancer, diabetes, arthritis, and dental caries are directly related to diet and nutrition. Much of the evidence comes from epidemiological investigations and some from laboratory animal studies and limited clinical observations.

Although there is no definitive scientific evidence of direct causation, there are various clues to indicate that the development of prudent dietary habits early in life and the continuation of these habits throughout life may minimize the incidence of the above diseases. Also, it appears that modern man's excessive caloric intake, relative to caloric expenditure, is directly associated with many chronic diseases.

Specifically, with respect to cancer, there is an increasing number of studies relating dietary and nutrient excesses, deficiencies, and imbalances to cancer development. For example, cancers of the stomach, colon, gastrointestinal tract, pancreas, and liver have been specifically correlated with certain dietary components.

Diet and nutrition are not only important in the etiology of cancer but they are also supportive procedures for the traditional modes of cancer therapy--surgery, radiotherapy, and chemotherapy. Preliminary laboratory investigations also point out that the use of diet and nutrition may be feasible for cancer therapy per se. Irrespective of the future role of nutrition in the

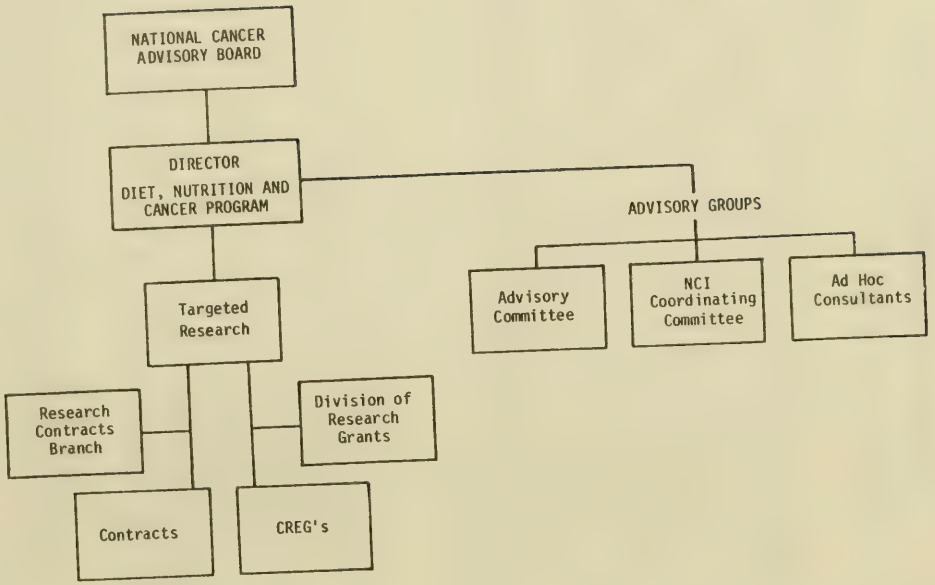

FIGURE 1. Organization of the Diet, Nutrition and Cancer Program

field of therapy, diet and nutrition will be important in improving the quality of life and in the ultimate rehabilitation of the cancer patient.

The Diet, Nutrition and Cancer Program is designed so that it:

- is a coordinated, multidisciplinary research program;
- is targeted towards specific goals and objectives;
- utilizes existing nutritional and dietary methodology and information;
- is oriented towards the development of practical and directly usable results;
- is responsive to the needs of all segments of the population including children who are often neglected; and
- disseminates information to the general scientific community and to the lay public.

STRUCTURE

The initial structure of the program emphasizes etiology and therapy as primary planning elements. Adjunct to this are the activities necessary to support, administer, and manage the program. The following presents a general overview of the nature and orientation of the program elements.

Etiology: This research element is oriented towards ascertaining the underlying causes and interrelationships among dietary and nutritional factors and cancer development. Information derived from etiological investigations will be utilized ultimately to develop prudent dietary recommendations to minimize the incidence of cancer and other diseases. Information will also provide the basis for developing new concepts for therapeutic regimens. Emphasis will be placed on ultimately defining desirable diets. Children will be a primary target for investigation because chronic diseases have relatively long latent periods and dietary habits are formed early in life.

Research in etiology will be conducted in the following areas:

Evolution: The objective of this research on dietary adaptation and animal models is to obtain data and information that will test the following hypothesis: If cancer, diet, and nutrition are related, the changes in man's dietary habits (and his physiological adaptation to these habits) will be accompanied by changes in his cancer experience. Examination will be made of the evolutionary determinants of dietary adaptation, of the natural dietary habits of man during his development, and animal models will be developed that might provide information and data on the effects of changing food habits.

Dietary excesses and deficiencies: This area includes acute and chronic studies of dietary alterations in man and animals to assess the effect of

various dietary components on carcinogenesis. Such factors as undernutrition or over nutrition and level of proteins, fats, carbohydrates, fiber, vitamins, minerals, and antioxidants will be studied.

Development of methods for assessing nutritional status: This area is oriented to the baseline compilation and analysis of information on dietary patterns and biochemical and metabolic parameters in various population groups.

Epidemiologic and dietary surveys: The objective of this research is to develop leads related to the causes of cancer. Leads will be developed using standard epidemiologic methodology in conjunction with dietary surveys using retrospective, prospective, and cross-sectional analyses.

Therapy: This research element is oriented towards the role of metabolic and dietary processes in the development of cancer and the use of dietary and nutritional supplementation in the support of traditional cancer treatment modalities--surgery, chemotherapy, and radiotherapy. The possibility of utilizing diet and nutrition as a direct therapeutic regimen is also to be evaluated. Various methods of increasing the food intake of anorectic cancer patients (adult and pediatric) will be investigated. The expected results to be derived from this element include the definition of dietary recommendations for optimal nutritional support, the development of suggested dietary intervention techniques to increase nutrient intake, the development of direct nutritional therapeutic solutions, and the development of cancer screening methods based on changes in metabolic processes. The overall improvement of the cancer patient's quality of life is the major goal of this research element. This will include assisting the cancer patient in coping with traditional therapeutic regimens, increasing the survival rates, and making rehabilitation easier. Emphasis will be placed on the pediatric patients because they have limited nutrient reserves for undergoing growth processes; from a psychological point of view, diet and proper nutrition could have a major impact on their ability to cope with therapy.

The following research areas are included in the therapy element:

Host-tumor competition and food intake determinants: The objective of this area is to focus on changes in metabolic processes which are produced by cancer in man. These changes could be in terms of protein, lipid, and energy metabolism and in terms of toxic substances produced by the tumor. Initial research emphasis will be placed on man and animal models in which tumors are early and the metabolic effects associated with cancer are interpretable. General areas for specific research include:

- anorexia and weight loss,
- depletion and redistribution of body compartments,
- energy and other nutrient problems, and
- processes by which the above occur.

Anorexia: Behavioral and food modification intervention. This area will include research in the area of the anorexia of malignancy. Studies will be undertaken on the alteration of taste and smell perception in the cancer patient and on the modification of food receptors and of food characteristics to improve caloric intake.

Artificial alimentation: The objectives of this research area are to:

- demonstrate that those cancer patients who normally suffer from malnourishment can achieve a better quality of life when brought into a normal metabolic state with artificial alimentation, and
- evaluate acute therapeutic results of current treatments in cancer patients who are in a normal metabolic state as a result of artificial alimentation.

In addition to the above major research elements, various activities will be carried out by the Diet, Nutrition and Cancer Program (DNCP) in the area of program management and support. These activities are necessary for:

- management and technical activities in support of the DNCP,
- program definition workshops for the DNCP,
- provision of ad hoc consultants for the technical and scientific monitoring of projects, and
- development of a technical information acquisition and dissemination system for the DNCP.

PROGRAM STATUS

This section presents an overview of the program status in terms of ongoing projects, information development and dissemination activities, coordinating efforts with other organizations, and a review of scientific and technical program definition workshops.

Ongoing Projects

At the present time, there are 34 ongoing contracts (six which will terminate during FY-77) and 9 CREG's sponsored by the DNCP. Proposals for contracts are reviewed by two DNCP scientific review committees. CREG applications are processed by the NIH Division of Research Grants and evaluated by ad hoc special study sections. Development processes for contracts and CREG's are shown in Figures 2 and 3, respectively.

Many of the projects, especially in the therapy area, are multi-institutional coordinated research efforts. In these, a common core protocol has been implemented by the participating investigators. Each investigator can also, within the specific constraints of his project workscope, budget, and defined objectives of the DNCP, undertake additional research. Therefore, by this approach, the targeted goals of the DNCP can be achieved while accommodating

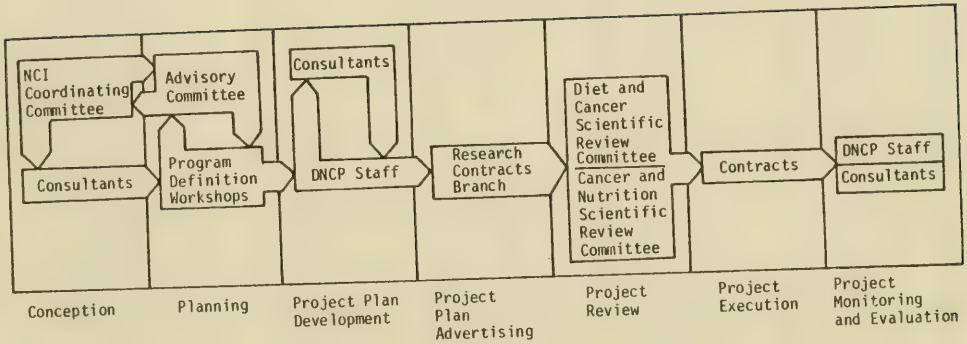

FIGURE 2. Development Process for Contracts

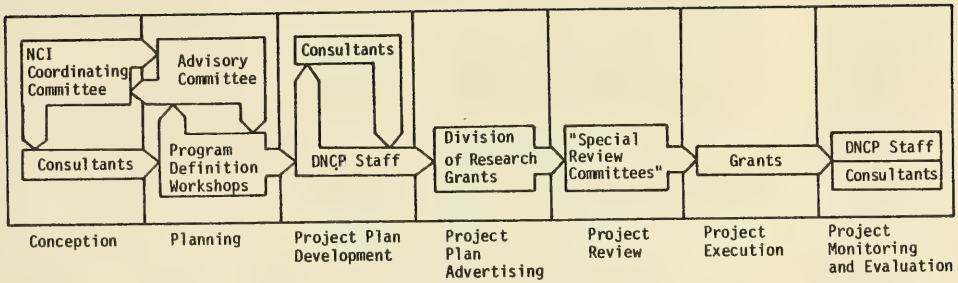

FIGURE 3. Development Process for Cancer Research Emphasis Grants

the basic research interests of the investigator. In addition, there are many common data elements and procedural links across the program. For example, the anorexia project will be able to assimilate data collected in the optimal nutritional support projects and those oriented to taste and smell. This will not only provide requisite information for assessing the validity of individual project results but will also greatly increase the benefit/cost ratio of the program's expenditures.

The role of the program staff in the multi-institutional projects, as well as in those research efforts involving extensive protocol development, has been to assist in coordination, experimental design, and methodological evaluation. (This is not only a staff function but also that of ad hoc program consultants.) The DNCP in most cases also serves as the statistical and data center for these projects.

Information Development and Dissemination

Besides the primary activity of assisting in the development and implementation of specific projects, the DNCP has also been involved in various information development and dissemination activities. These activities not only involve the preparation of documents for the scientific community but also for the lay public and the cancer patient. The program, with the assistance of the NCI International Cancer Research Data Bank, is presently in the process of developing the following program documents and services:

- State-of-the-Art Reviews: The objective of these documents is to provide the research community current information on the relationship of nutrient classes to cancer etiology and the use of nutrition in cancer therapy and rehabilitation.

- Cancergram: This current awareness service will help maintain scientific knowledge in rapidly changing disciplines.

- Cancerprojects: These documents would provide a listing of ongoing projects in both therapy and etiology for both the U.S. and foreign countries.

The DNCP is developing various innovative approaches for presenting scientific and technical information relevant to nutrition and cancer, and utilizing the literature as a resource planning tool. As an initial approach, approximately 400 journals applicable to the program were structured into eight categories representing the following areas: anthropology, behavioral science, biochemistry, cancer, hygiene and public health, medicine, nutrition and dietetics, and overall composite category. A computer based citation analysis on the journals was performed for the year 1975. (Approximately 1,200,000 citations were analyzed.) The output is in the form of "mappings" which relate specific clusters of research within a field. The relationship of the various clusters are displayed as well as the intensity of the relationship. It appears that this type of analysis can prove most useful in the planning of the DNCP, identifying possible research gaps, and ensuring that the program capitalizes on existing lines of research.

In addition, the DNCP has assisted in the preparation of two handbooks oriented to the parents of pediatric cancer patients and to adult cancer patients and their families.

Coordination

The DNCP, based on a recommendation from the Advisory Committee, has over the past year established contacts and working relationships with other organizations sponsoring nutrition research. The purpose of this effort is to ensure that the program is fully cognizant of related ongoing research, and to provide information for planning purposes, so that the DNCP can capitalize on research findings.

Lists of the Diet, Nutrition and Cancer Program Advisory Committee, the Diet and Cancer Scientific Review Committee, the Cancer and Nutrition Scientific Review Committee, and the DNCP consultants follow:

- Diet, Nutrition and Cancer Program Advisory Committee

  Mrs. Elaine Adams*, New York, New York
  Dr. William Darby, Nutrition Foundation
  Dr. Stanley Dudrick, University of Texas Medical School at Houston
  Dr. Peggy C. Fry, University of Texas at Dallas
  Mr. Larry Garfinkel, American Cancer Society
  Dr. Gio B. Gori (Executive Secretary), National Cancer Institute
  Dr. Willis A. Gortner, American Institute of Nutrition
  Dr. Richard A. Greenberg*, Swift and Company
  Ms. Beverly McGaughy, Washington Area Candlelighters
  Dr. Harold Sandstead, U. S. Department of Agriculture
  Dr. Athanasios Theologides, University of Minnesota Medical School
  Dr. William G. Thurman, University of Oklahoma
  Dr. Myron Winick, Columbia University
  Dr. Gerald Wogan (Chairman), Massachusetts Institute of Technology
  Dr. Ernst L. Wynder, American Health Foundation
  Dr. Harold Amos (Liaison, NCAB), Harvard University
  Dr. Jonathan E. Rhoads (Ex-Officio), University of Pennsylvania School of Medicine

- Diet and Cancer Scientific Review Committee

  Dr. Donald Austin, California Department of Health
  Dr. Myrtle Brown, National Research Council
  Dr. Wanda L. Chenoweth, Michigan State University
  Dr. Edward M. Copeland (Chairman), University of Texas
  Dr. Marilyn Crim, Massachusetts Institute of Technology
  Dr. Gio B. Gori (Executive Secretary), National Cancer Institute
  Dr. Ronald Hunt*, Harvard Medical School
  Dr. Murray Kaplan, Rutgers-The State University
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Dr. Charlotte Mistretta, University of Michigan
Dr. W. E. C. Moore, Virginia Polytechnic Institute and State University
Dr. Malden C. Nesheim, Cornell University
Dr. Peter L. Pellett, University of Massachusetts
Dr. David P. Rose, University of Wisconsin
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Ms. Carol Hunerlach, Consulting Nutritionist, Ardmore, Maryland
Dr. Norge W. Jerome, University of Kansas
Dr. Jerome L. Knittle, Mount Sinai School of Medicine
Dr. O. Neal Miller, Hoffmann-LaRoche, Inc.
Dr. David Paige, Johns Hopkins University
Dr. Maurice Shils, Memorial Hospital
Dr. Robert W. Sussman, Washington University
Dr. John E. Vanderveen, Food and Drug Administration

- Diet, Nutrition and Cancer Program Consultants

Elaine Adams, DNCP Advisory Committee
Siamak A. Adibi, Montefiore Hospital
William Admirand, San Francisco General Hospital
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James R. Allen, Jr., University of Wisconsin
Bruce N. Ames, University of California
Harold Amos, Harvard University
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Gary Beauchamp, Monell Chemical Senses Center
Raymond B. Baggs, Cornell University
Peter Baillie, Queen Elizabeth College
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Abby Bloch, Memorial Sloan-Kettering Cancer Center
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Gilbert B. Bradham, Medical University of South Carolina
Joseph Brand, Monell Chemical Senses Center
Murray F. Brennan, National Cancer Institute

Myron Brin, Hoffmann-LaRoche, Inc.
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David G. Cornwell, Ohio State University
Giovanni Costa, Private Physician
Marilyn Crim, Massachusetts Institute of Technology

Loring B. Dales, Kaiser Permanente
William J. Darby, Nutrition Foundation
John Davis, University of Illinois
William D. DeWys, Northwestern University
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Patricia Dinkelacher, Consulting Dietitian
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Eric Eisenstadt, Harvard University

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Josef E. Fischer, Harvard Medical School
W. Gary Flamm, National Cancer Institute
Samuel J. Fomon, University of Iowa
Frederick J. Francis, University of Massachusetts
Carol Frankmann, University of Texas
Reva Frankle, Weight Watchers International, Inc.
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Emil J. Freireich, University of Texas
Douglas Frost, Consultant, Nutrition Biochemistry
Peggy C. Fry, University of Texas

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Annette Gormican, University of Wisconsin
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Saxon Graham, State University of New York
Richard A. Greenberg, Swift and Company
Harry L. Greene, Vanderbilt University
Russ C. Greer, University of Tennessee
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Ronald D. Hunt, New England Regional Primate Research Center

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Norge W. Jerome, University of Kansas
Henry A. Jordan, University of Pennsylvania

Harold Kahn, Johns Hopkins University
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Bette Kalish, Consulting Technical Writer
Joseph J. Kanapka, National Institutes of Health
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Morley R. Kare, University of Pennsylvania
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David Kritchevsky, Wistar Institute of Anatomy and Biology
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Jack Metcoff, University of Oklahoma
Walter H. Meyer, Proctor and Gamble Company
Neal O. Miller, Hoffmann-LaRoche, Inc.
Sydney Mirvish, Eppley Institute for Research in Cancer

Charlotte Mistretta, University of Michigan School of Dentistry
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Mary Monk, New York Medical College
W. E. C. Moore, Virginia Polytechnic Institute
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Irvin Plough, National Cancer Institute
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Michael Prival, Food and Drug Administration
Leon Prosky, Food and Drug Administration

Thomas H. Randall, Brown University
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Jonathan E. Rhoads, University of Pennsylvania
Miguel Riella, University of Washington
Basil Rifkind, National Heart and Lung Institute
Richard S. Rivlin, Columbia University
Robert Rizek, U. S. Department of Agriculture
Willard L. Roberts, Beatrice Research Laboratories
John R. K. Robson, Medical University of South Carolina
Daphne Roe, Cornell University
Dale R. Romsos, Michigan State University
David P. Rose, University of Wisconsin
Morris H. Ross, Institute for Cancer Research
Daniel Rudman, Emory University

Z. I. Sabry, Nutrition Research Consultants
Gerald Salen, New Jersey Medical School
Harold H. Sandstead, U. S. Department of Agriculture
Sam Sarof, Institute for Cancer Research
Arnold Schaefer, Swanson Center for Nutrition
Howard Schneider, University of North Carolina
David Schottenfeld, Memorial Hospital
Norman R. Scott, Cornell University
Belding H. Scribner, University of Washington
Bruce Semple, Johnson and Johnson Baby Products Division

Raymond Shamberger, Cleveland Clinical Foundation
Maurice Shils, Memorial Hospital
Jo Ann Simon, University of Texas
Tipor Sipos, Johnson and Johnson Research
Kathryn W. Smith, Illinois State University
Leland Smith, University of Texas Medical Branch
Fred L. Snyder, Oak Ridge Associated Universities
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Stephen P. Stephen, University of Texas
William Stini, University of Arizona
John T. Stitt, John B. Pierce Foundation
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Robert W. Sussman, Washington University
Daris R. Swindler, University of Washington

Melvin Tefft, Rhode Island Hospital
Athanasios Theologides, University of Minnesota
Alexander E. Thomas, Dwight P. Joyce Research Center
William G. Thurman, University of Oklahoma
Jeanne Tillotson, National Heart and Lung Institute
Samuel B. Tove, North Carolina State University
Dorothea Turner, American Dietetic Association

John E. Vanderveen, Food and Drug Administration
Benjamin VanDuren, New York University
Willard J. Visek, University of Illinois
Joseph J. Vitale, Boston University

Leonard Waldman, Abbott Laboratories
Christine Waterhouse, Strong Memorial Hospital
L. W. Wattenberg, University of Minnesota
John Weisburger, Naylor-Dana Institute
James Whitehead, Veterans Administration Hospital
Lars H. Wiederman, Swift and Company
Manfred Wilk, University of Frankfurt
Douglas W. Wilmore, U. S. Army Institute of Surgical Research
Robert B. Wilson, Washington State University
Myron Winick, Columbia University
Gerald Wogan, Massachusetts Institute of Technology
George Wolf, Massachusetts Institute of Technology
Ernst L. Wynder, American Health Foundation

Norman N. Yoshimura, McGaw Laboratories
Charlotte Young, Consulting Nutritionist
Vernon Young, Massachusetts Institute of Technology

Workshops

The primary method of formulating proposals for research projects is through the utilization of program definition workshops. These workshops are oriented to the following objectives:

- reviewing the state-of-the-art in a given research area;

- relating the state-of-the-art to the needs of the DNCP; and
- delineating specific projects including:
  - experimental hypotheses and their relationship to the topical area,
  - preferred experimental approaches and their rationale with procedural caveats, and
  - estimated levels of effort and an indication of requisite scientific and technical qualifications of personnel.

As of June 1, 1977, the following FY'77 workshops have been held:

Workshop	Date
Studies on Cancer of the Stomach and Upper Gastrointestinal Tract	August 3, 1976
Use of Oral Solutions in the Treatment of Cancer Patients	August 17, 1976
The Possible Role of Cholesterol in Carcinogenesis	August 19, 1976
Total Parenteral Nutrition--Delivery Systems, Hardware, and Techniques	August 25, 1976
Nutrition Education	August 26, 1976 October 8, 1976
Digestive Enzyme Replacement Therapy	August 31, 1976
Role of Vitamins in Carcinogenesis	September 30, 1976 November 11, 1976
Anutrient Compounds and Carcinogenesis	November 12, 1976
Efficacy of *In Vitro* Tests as Presumptive Test for Carcinogenesis of Dietary Compounds	November 17, 1976
Energy Metabolism in Cancer	November 22-23, 1976
Design of Animal Feeding Experiments in Carcinogenesis: Specifications of Dietary Fat	December 3, 1976
Nutritional Anthropology/Nutritional Epidemiology	April 28, 1977

Diet, Nutrition and Cancer Program Current Contracts - FY'77

Organization	Project Title
Albert Einstein College of Medicine Bronx, N.Y. NO1-CP-65788	Correlation of Epidemiologic and Metabolic Parameters
American Health Foundation New York, N.Y. NO1-CP-65818	Correlation of Epidemiologic and Metabolic Parameters
American Institutes for Research Cambridge, Mass. NO1-CP-65781	Literature Study on Primitive Populations in Relation to Diet
American Institutes for Research Cambridge, Mass. NO1-CP-65793	Literature Study to Evaluate Health Parameters in Various Human Populations in Relation to Diet
Boston University Medical Center Boston, Mass. NO1-CP-65869	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 4)
Boston University Medical Center Boston, Mass. NO1-CP-65872	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 7)
Children's Hospital of Los Angeles Los Angeles, Calif. NO1-CP-65828	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient
Children's Hospital Medical Center Boston, Mass. NO1-CP-65826	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient
Children's Hospital at Stanford Palo Alto, Calif. NO1-CP-65835	Anorexia in Adult and Pediatric Cancer Patients
Children's Orthopedic Hospital and Medical Center Seattle, Wash. NO1-CP-65790	Evaluation of the Role of Learned Food Aversion in the Cancer Patient
Emory University School of Medicine Atlanta, Ga. NO1-CP-65873	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 8)

Organization	Project Title
Enviro Control, Inc. Rockville, Md. NO1-CP-65759	Development, Management and Support Services to the Diet, Nutrition and Cancer Program
Georgetown University Washington, D.C. NO1-CP-65838	Anorexia in Adult and Pediatric Cancer Patients
Health Research, Inc. Buffalo, N.Y. NO1-CP-65867	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 2)
Institute for Scientific Information Philadelphia, Pa. NO1-CP-65772	Review and Analysis of Categorical Citation Information Relevant to the Diet, Nutrition and Cancer Program
Massachusetts Institute of Technology Cambridge, Mass. 02139 NO1-CP-65865	Role of Pre-Formed Dietary Purines and Pyrimidines in the Genesis of Cancer of the GI Tract
Medical College of Virginia Richmond, Va. NO1-CP-75881	Studies of Differential Nutritional Requirements by Host and Tumor as the Basis for Dietary Treatment of Cancer
Medical University of South Carolina Charleston, S.C. NO1-CP-65780	Literature Study on Indicators of Health and Nutritional Status with Emphasis on Primitive Populations
Memorial Hospital for Cancer and Allied Diseases New York, N.Y. NO1-CP-65829	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient
Midwest Research Institute Kansas City, Mo. NO1-CP-65787	Application of Behavior Modification Techniques in the Treatment of Anorexia in the Cancer Patient
New England Deaconess Hospital Boston, Mass. NO1-CP-65870	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 4)
Northwestern University Chicago, Ill. NO1-CP-65779	Anorexia in Adult and Pediatric Cancer Patients

Organization	Project Title
Research Foundation, State University of New York Albany, N.Y. NO1-CP-65834	Anorexia in Adult and Pediatric Cancer Patients
Ruhl Associates Alexandria, Va. NO1-CP-65789	Identification of Past, Ongoing and Future Dietary and Nutritional Surveys and Cancer Epidemiology Studies
Stanford University Stanford, Calif. NO1-CP-65825	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient
State University of New York at Buffalo Albany, N.Y. NO1-CP-65868	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 3)
University of North Carolina Chapel Hill, N.C. NO1-CP-657880	Development of a Guidebook for Inclusion of Dietary and Anthropometric Parameters in Cancer Epidemiology Studies
University of Pennsylvania Philadelphia, Pa. NO1-CP-65802	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 1)
University of Rochester Rochester, N.Y. NO1-CP-65833	Gustatory (Taste) Evaluation of Cancer Patients
University of Tennessee Memphis, Tenn. NO1-CP-65786	Studies of Differential Nutritional Requirements by Host and Tumor as the Basis for Dietary Treatment of Cancer
University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute Houston, Tex. NO1-CP-65794	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient
University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute Houston, Tex. NO1-CP-65866	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 6)

Organization	Project Title
University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute Houston, Tex. NO1-CP-65871	Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 5)
University of Wisconsin Madison, Wisc. NO1-CP-65784	Survey of Dietetic Practices and Procedures used in Feeding Cancer Patients

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP-65788)

Title: Correlation of Epidemiologic and Metabolic Parameters

Contractor's Project Director: Dr. Seymour L. Romney

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objectives of this project are to (1) investigate epidemiological and nutritional variables (especially vitamin A intake) which may differentiate women with positive or suspicious Pap smears from women with negative Pap smears; and (2) investigate possible relationships between the development of cervical cancer or dysplasia and vitamin A receptor content and/or binding in cervical tissue.

Major Findings: Preliminary analysis of 24-hour food recall data indicates a large difference in dietary vitamin A between cases and controls. Studies of the presence and total number of binding sites in plasma and tissue are inconclusive at present.

Significance to Biomedical Research and the Program of the Institute: Previous studies have shown that vitamin A is important for normal growth and proper cellular differentiation of epithelial cells. Cancer of the cervix is the abnormal growth of the epithelial cells of cervical tissue; hence this study is designed to investigate dietary vitamin A and the mechanism of vitamin A uptake in women with cervical cancer or dysplasia and normal controls.

Proposed Course: The optional year of this contract will emphasize the dietary aspects of the study. Biochemical investigations will receive lower priority and be initially concerned with validation of the assay technique.

Date Contract Initiated: June 30, 1976

Current Award Level: in negotiation

AMERICAN HEALTH FOUNDATION (NO1-CP-65818)

Title: Correlation of Epidemiologic and Metabolic Parameters

Contractor's Project Director: Dr. Ernst L. Wynder

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Part I (colon cancer segment) - The objectives of this segment of the project are to (1) collect general background data such as nutritional habits, demographic characteristics, and diagnostic and histopathological data; (2) collect similar data on control patients with diseases not etiologically associated with colon cancer and to determine if differences exist in dietary fat consumption between cases and controls; (3) study the cholesterol conversion pattern by fecal microflora from patients with colon cancer and from controls selected from the groups mentioned above; and (4) study the effect of varying cooking time of meat in a high fat meat diet on the composition of fecal bile acids and cholesterol metabolites and on fecal bacterial β-glucuronidase, 7α-dehydrogenase, and cholesterol dehydrogenase activities.

Part II (prostate cancer segment) - The objectives of this project segment are to (1) investigate the hormone profiles and pituitary or testicular responsiveness of healthy men and prostatic cancer cases in low-risk populations versus high-risk populations; (2) investigate the profiles in healthy adolescents in each of the population groups; (3) obtain dietary data and estimates of composition of food intake in each of the population groups; and (4) determine the effect of exchanging Western for vegetarian diets in each of the groups mentioned.

Major Findings: Part I - Preliminary findings indicate that cholesterol conversion activity is higher in colon cancer patients compared to healthy controls. The diet modification study is under way but, as yet, no conclusions can be drawn.

Part II - This segment has gotten off to a slow start, partly due to arrangements which had to be made with doctors in other countries. In the dietary intervention study, preliminary findings indicate possible changes in urinary estradiol, but sample sizes are too small for a more specific statement.

Significance to Biomedical Research and the Program of the Institute: Part I - The study is expected to produce a large amount of data which may relate dietary or other factors to the development of colon cancer. Specific studies will investigate the role of dietary animal fat on fecal constituents in an effort to find metabolic mechanisms for the development of colon cancer and related diseases and to identify bacterial and chemical indicators.

Part II - Although the incidence of prostatic cancer is known to vary according to ethnic group and geographic area, the factors responsible for the differences are unknown. The number of latent foci, which are

regarded as sites for the future development of prostatic cancer, is similar to low-risk versus high-risk populations. This study is an attempt to find hormonal differences which may be correlated with increased risk of prostatic cancer and to determine the effect of vegetarian versus Western diets on hormone profiles and pituitary responsiveness.

Proposed Course: Part I - Studies will continue as planned. This includes completion of patient entry and data collection, compilation and analysis of data, and a final report.

Part II - Studies will continue as planned. This includes completion of patient entry and data collection, compilation and analysis of data, and a final report.

Date Contract Initiated: June 30, 1976

Current Annual Level: $195,452

AMERICAN INSTITUTES FOR RESEARCH (NO1-CP-65781)

Title: Literature Study on Primitive Populations in Relation to Diet

Contractor's Project Director: Dr. Kristina Cannon-Bonventre

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Project objective was to conduct a comprehensive literature review examining the interrelationships of dietary factors and cancer in subsistence and transitional societies. The hypothesis of this study was that (1) some elements in the diets of various human groups may create secondary or even tertiary changes that predispose members of those groups to cancer, and (2) other elements may provide a particular group or subgroup with a degree of protection from cancer. The overall objectives were to find, evaluate, compile, and synthesize extant literature relevant to this hypothesis.

Major Findings: Based on an extensive literature review, the investigator proposed 16 populations for further study. Additionally, methodologies that have been particularly valuable in such research were delineated.

Significance to Biomedical Research and the Program of the Institute: This project provided the Diet, Nutrition and Cancer Program with bibliographic listings, critical reviews of important articles, and summarizations of what is known about the relationship between diet and cancer in particular populations.

Proposed Course: A workshop sponsored by the DNCP will evaluate and prioritize the research recommendations generated by this report.

Date Contract Initiated: June 30, 1976

Current Annual Level: $47,925 (This project was funded in FY'76 and completed in FY'77.)

AMERICAN INSTITUTES FOR RESEARCH (NO1-CP-65793)

Title: Literature Study to Evaluate Health Parameters in Various Human Populations in Relation to Diet

Contractor's Project Director: Dr. Kristina Cannon-Bonventre

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The aim of this study was to conduct a comprehensive review of literature concerning the interrelationships between dietary factors, health parameters, and cancer in high-risk and low-risk populations. Three types of studies were included in the bibliographic review: (1) studies that concentrate on the health and dietary parameters of populations with an unusually high or low reported incidence of cancer; (2) studies of populations in transition, i.e., those that now contain subpopulations partially or wholly transformed by rapid technological change or those to which subpopulations have migrated, resulting in dietary changes (such populations are especially important as "natural laboratories" in which the effects of dietary and environmental changes on human disease patterns can be seen); and (3) studies that are models for the investigation of diet and health prarameters, because of excellence of research design, data collection, and/or analysis.

Major Findings: The Diet, Nutrition and Cancer Program was provided with approximately 1,500 bibliographic listings of articles, books, and reports of unpublished studies dealing with diet, cancer, and health parameters. From these listings, approximately 275 were selected for annotation. In addition, 15 populations were selected as the subjects of extended reviews covering major information documented in the literature to date and including detailed recommendations for future work on the populations.

Significance to Biomedical Research and the Program of the Institute: This review has provided the DNCP with a foundation for future investigative efforts.

Proposed Course: A workshop will be held to develop specific research ideas based on the recommendations developed under this contract.

Date Contract Initiated: June 30, 1976

Current Annual Level: $48,588 (This project was funded in FY'76 and completed in FY'77.)

BOSTON UNIVERSITY MEDICAL CENTER (NO1-CP-65869)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 4--Radiotherapy, Abdomen and Pelvis Malignancies)

Contractor's Project Director: Dr. William P. Steffee

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with abdomen and pelvis malignancies to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $83,179

BOSTON UNIVERSITY MEDICAL CENTER (NO1-CP-65872)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 7--Chemotherapy, Carcinoma of the Breast)

Contractor's Project Director: Dr. William P. Steffee

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with breast malignancies to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

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Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is underway. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $83,329

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CP-65828)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient

Contractor's Project Director: Dr. Daniel M. Hays

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is designed to establish a relationship between the nutrition of pediatric cancer patients and the effectiveness of multi-modality therapy. More specifically, through a randomized prospective study, the project will attempt to determine whether patients provided with total parenteral nutrition demonstrate a greater tolerance for antineoplastic therapy and a better therapeutic response than similar patients receiving standard nutritional regimens.

Major Findings: This study will provide an opportunity to test the feasibility and practicality of using total parenteral nutrition to support traditional modes of anticancer therapy. It will be studied as a means of increasing survival time and improving the quality of pediatric cancer patients' lives during and after therapy. If it is found that adequate nutritional status widens therapeutic margins without proliferating tumorous growth, therapeutic regimens can be improved.

Significance to Biomedical Research and the Program of the Institute: The project is designed to assess the possible benefits of total parenteral nutrition and to answer the following questions: (1) Does optimal nutritional support enhance tumor response to radiation therapy or to various chemotherapeutic regimens? (2) Does the pediatric patient with adequate nutrition before treatment respond better to oncologic therapy? Is this response similar to that obtained in the patient who has never experienced weight loss secondary to tumor burden? (3) Can the toxic side effects of radiotherapy and chemotherapy be reduced, eliminated, or prevented by optimal nutritional support? If so, can a higher dose of therapy be administered because of reduced toxicity? Does increasing the dose result in better tumor response? If normal tissue injury does occur, does the nutritional support result in more rapid healing and decrease long-term sequelae? (4) Is tumor growth enhanced or inhibited by optimal nutritional support?

Proposed Course: Thirty pediatric cancer patients undergoing similar treatment modalities for abdomen and pelvic tumors are, or will be, involved in the study. Patients are assessed before, during, and after the administration of antineoplastic therapy, and their responses to therapy and nutritional support are evaluated. The experimental period (phase I) is completed within 1-year of admission to the study while the follow-up period (phase II) lasts an additional 2 years.

Date Contract Initiated: June 30, 1976

Current Annual Level: $62,749

CHILDREN'S HOSPITAL MEDICAL CENTER (NO1-CP-65826)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient

Contractor's Project Director: Dr. Robert Filler

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is designed to establish a relationship between the nutrition of pediatric cancer patients and the effectiveness of multi-modality therapy. More specifically, through a randomized prospective study, the project will attempt to determine whether patients provided with total parenteral nutrition demonstrate a greater tolerance for antineoplastic therapy and a better therapeutic response than similar patients receiving standard nutritional regimens.

Major Findings: This study will provide an opportunity to test the feasibility and practicality of using total parenteral nutrition to support traditional modes of anticancer therapy. It will be studied as a means of increasing survival time and improving the quality of pediatric cancer patients' lives during and after therapy. If it is found that adequate nutritional status widens therapeutic margins without proliferating tumorous growth, therapeutic regimens can be improved.

Significance to Biomedical Research and the Program of the Institute: The project is designed to assess the possible benefits of total parenteral nutrition and to answer the following questions: (1) Does optimal nutritional support enhance tumor response to radiation therapy or to various chemotherapeutic regimens? (2) Does the pediatric patient with adequate nutrition before treatment respond better to oncologic therapy? Is this response similar to that obtained in the patient who has never experienced weight loss secondary to tumor burden? (3) Can the toxic side effects of radiotherapy and chemotherapy be reduced, eliminated, or prevented by optimal nutritional support? If so, can a higher dose of therapy be administered because of reduced therapy? If normal tissue injury does occur, does the nutritional support result in more rapid healing and decrease long-term sequelae? (4) Is tumor growth enhanced or inhibited by optimal nutritional support?

Proposed Course: Twenty-four pediatric cancer patients undergoing similar treatment modalities for abdomen and pelvic tumors are, or will be, involved in the study. Patients are assessed before, during, and after the administration of antineoplastic therapy, and their responses to therapy and nutritional support are evaluated. The experimental period (phase I) is completed within 1-year of admission to the study while the follow-up period (phase II) lasts an additional 2 years.

Date Contract Initiated: June 30, 1976

Current Annual Level: $34,372

CHILDREN'S HOSPITAL AT STANFORD (NO1-CP-65835)

Title: Anorexia in Adult and Pediatric Cancer Patients

Contractor's Project Director: Ms. Joy N. Dutcher

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is part of a multidisciplinary, multi-institutional cooperative effort and deals with the causes and effects of anorexia in pediatric cancer patients. Anorexia is considered a significant cause of morbidity in cancer patients. Researchers are collecting detailed dietary, nutritional, anthropometric, socioeconomic, psychological, metabolic, and physiological data on 75 pediatric cancer patients and 75 matched sibling controls. Both retrospective and prospective data are being collected.

Major Findings: Anorexia develops as a complication of malignancy in a significant proportion of cancer patients. In some, anorexia may be related to interference with intestinal function, while in others anorexia may be related to the endocrine or hormonal effects of a neoplasm. However, in the majority of patients, such obvious factors are not present, and anorexia is often largely unexplained. It is anticipated that the findings of these studies will help to fill this gap in knowledge.

Significance to Biomedical Research and the Program of the Institute: This effort will pave the way for future research, both of the altered metabolic processes associated with specific types of cancer and of the development of methods of increasing food intake; or perhaps it will lead to a coordinated study of the two. The project may also result in the rapid development of intervention protocols that offer immediate opportunities of supporting the traditional modes of cancer treatment (surgery, radiotherapy, and chemotherapy) and of increasing cancer survival rates.

Proposed Course: The information collected in this investigation will be analyzed to develop hypotheses on the causes of anorexia and the interrelationships between anorexia, cancer type, treatment modality, and associated phenomena.

Date Contract Initiated: June 30, 1976

Current Annual Level: $119,518

CHILDREN'S ORTHOPEDIC HOSPITAL AND MEDICAL CENTER (NO1-CP-65790)

Title: Evaluation of the Role of Learned Food Aversion in the Cancer Patient

Contractor's Project Director: Dr. Ilene Bernstein

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is designed to determine whether learned food aversions are acquired by patients treated with therapeutic regimens that induce gastrointestinal toxicity. If learned food aversions are found, investigators will determine whether such aversions contribute substantially to the anorexia and cachexia frequently afflicting cancer patients.

Major Findings: Laboratory studies are aimed at determining the effects of repeated administration of gastrointestinal toxic drugs on overall food intake and body weight in tumor bearing and control rats. The effect of tumor growth on the animals' propensity to form conditioned taste aversions is being evaluated.

Significance to Biomedical Research and the Program of the Institute: It is anticipated that, using the results of these experiments, researchers can develop strategies for circumventing or minimizing learned food aversions associated with cancer treatment.

Proposed Course: Approximately 80 pediatric and adult patients will be studied during the course of this project. Questionnaires and interviews aimed at detecting newly acquired food aversions in conjunction with chemotherapy will be administered.

Date Contract Initiated: June 30, 1976

Current Annual Level: $49,168

EMORY UNIVERSITY SCHOOL OF MEDICINE (NO1-CP-65873)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 8--Chemotherapy, Adenocarcinoma of the Colon)

Contractor's Project Director: Dr. Daniel W. Nixon

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with adenocarcinoma of the colon to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $31,444

ENVIRO CONTROL, INC. (NOI-CP-65759)

Title: Development, Management and Support Services to the Diet, Nutrition and Cancer Program

Contractor's Project Director: Mr. Jack C. Fensterstock

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this contract is to provide support services to the Diet, Nutrition and Cancer Program, including (1) monitoring ongoing research projects; (2) formulation of project plans; (3) development of study protocols and experimental designs; (4) design of forms, procedures, and data quality control techniques for clinical trials; (5) data processing and statistical analysis; (6) organization and coordination of meetings and development of agendas; (7) background reviews and literature searches; and (8) development of program related materials for dissemination to both the scientific community and the lay public.

Major Findings: As shown in the objectives, this contract is for support and management, not research per se.

Significance to Biomedical Research and the Program of the Institute: The contractor has assisted in the development of the Diet, Nutrition and Cancer Program based on information furnished by the DNCP Director and Advisory Committee. Responsibilities include participation in program planning activities.

Proposed Course: The contractor will continue present services in its role under a support contract agreement.

Date Contract Initiated: December 1, 1975

Current Annual Level: $569,043

GEORGETOWN UNIVERSITY (NO1-CP-65838)

Title: Anorexia in Adult and Pediatric Cancer Patients

Contractor's Project Director: Dr. Robert I. Henkin

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project, part of a multidisciplinary, multi-institutional cooperative effort, deals with the causes and effects of anorexia in adult cancer patients. Anorexia is considered a significant cause of morbidity in cancer patients. Researchers are collecting detailed dietary, nutritional, anthropometric, socioeconomic, psychological, metabolic, and physiological data on 100 adult cancer patients. Both retrospective and prospective data are being collected.

Major Findings: Anorexia develops as a complication of malignancy in a significant proportion of cancer patients. In some, anorexia may be related to interference with intestinal function, while in others anorexia may be related to the endocrine or hormonal effects of a neoplasm. However, in the majority of patients, such obvious factors are not present and anorexia is often largely unexplained. It is anticipated that the findings of these studies will help to fill this gap in knowledge.

Significance to Biomedical Research and the Program of the Institute: This effort will pave the way for future research, both of the altered metabolic processes associated with specific types of cancer and of the development of methods of increasing food intake; or perhaps it will lead to a coordinated study of the two. The project may also result in the rapid development of intervention protocols that offer immediate opportunities of supporting the traditional modes of cancer treatment (surgery, radiotherapy, and chemotherapy) and of increasing cancer survival rates.

Proposed Course: The information collected in this investigation will be analyzed to develop hypotheses on the causes of anorexia and the interrelationships between anorexia, cancer type, treatment modality, and associated phenomena.

Date Contract Initiated: June 30, 1976

Current Annual Level: $31,507

HEALTH RESEARCH, INC. (NO1-CP-65867)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 2--Surgery, Head and Neck Malignancies)

Contractor's Project Director: Dr. Kumao Sako

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with head and neck malignancies to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $21,841

INSTITUTE FOR SCIENTIFIC INFORMATION (NO1-CP-65772)

Title: Review and Analysis of Categorical Citation Information Relevant to the Diet, Nutrition and Cancer Program

Contractor's Project Director: Dr. Henry Small

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objectives of this project have been two-fold: (1) To identify highly regarded scientists as potential participants in DNCP workshops and review committees. (2) To map fields of research related to the DNCP to understand the relationship of research areas and thereby provide a guide for future program planning.

Major Findings: Eight special citation data bases were created for the following fields: anthropology, behavioral sciences, biochemistry, cancer, hygiene and public health, medicine, nutrition and dietetics, and an overall category which is a composite of all the others. Highly cited document and author lists were created for each category, and a cluster analysis of each generated a total of 4,887 clusters. The largest clusters were identified and named, and those remaining were scanned for areas relevant to the Diet, Nutrition and Cancer Program.

Significance to Biomedical Research and the Program of the Institute: The listings have been categorized according to topical and specialty areas that are relevant to the program, in order to assist in identifying new areas for research, consultants who can be utilized in the future, scientists who might participate in DNCP sponsored workshops, and information on foreign researchers and institutions.

Proposed Course: This project was completed October 9, 1976.

Date Contract Initiated: May 10, 1976

Current Annual Level: $18,870 (This project was funded and completed in FY'76.)

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-65865)

Title: Role of Pre-Formed Dietary Purines and Pyrimidines in the Genesis of Cancer of the GI Tract

Contractor's Project Director: Dr. Hamish Munro

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Several recent studies have emphasized the possibility of a nutritional and dietary influence on carcinogenesis. This study is oriented towards further definition of suspected dietary links to cancer, through study of the metabolism of specific compounds in relation to cancer incidence. Persuasive evidence has emerged that the mucosa of the small intestine lacks the capacity to manufacture purine based adenine and quanine de novo. These compounds may be provided either by the blood or, more likely, from purine bases liberated during the course of digestion and absorbed through the mucosa. The objective of this study is to determine the ability of the small intestine to synthesize purines and pyrimidines de novo and to investigate the roles of pre-formed dietary purines and pyrimidines in the carcinogenesis of the small and large intestines.

Major Findings: This project began October 1, 1976. No major findings are presently available.

Significance to Biomedical Research and the Program of the Institute: The observed absence or suppression of the de novo pathway in small intestinal mucosa may be important to the genesis and distribution of cancer in the intestinal tract. Perhaps the hypothesized inability of this mucosa to produce purines accounts for the scarcity of neoplasms in this area, due to a restraint on tumorous growth through limitation of purine supply.

Proposed Course: The capacity of the mucosa to synthesize purine nucleotides and pyrimidine nucleotides, the dietary relationships, and the relevance of these questions to carcinogenesis will be examined in this study.

Date Contract Initiated: October 1, 1976

Current Annual Level: $14,376

MEDICAL COLLEGE OF VIRGINIA (NO1-CP-75881)

Title: Studies of Differential Nutritional Requirements by Host and Tumor as the Basis for Dietary Treatment of Cancer

Contractor's Project Director: Dr. William L. Banks, Jr.

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this project is to evaluate the hypothesis that controlling the blood levels of a specific amino acid required by a particular glioblastoma will limit tumor growth without harming the host. Specifically, it is designed to (1) determine whether direct nutritional therapy alters the rate of brain tumor growth and can be used as a treatment modality; (2) determine whether direct nutritional therapy and concomitant antineoplastic therapy result in synergistic benefits; and (3) determine how direct nutritional therapy affects the host-tumor response and other associated parameters such as host-immune response. This project is a multi-institutional cooperative effort.

Major Findings: Chemotherapy and radiation therapy protocols have generally been unsuccessful in significantly prolonging the useful life of the brain cancer patient. Thus a nutritional approach to therapy, following surgical reduction of the tumor burden, is an important avenue for investigation. The project will define the feasibility of using direct nutritional therapy to treat other malignant tumor types.

Significance to Biomedical Research and the Program of the Institute: Success with this therapy modality would be an important breakthrough in the field of cancer treatment, which could lead to a significant increase in the survival rates of individuals with glioblastoma tumors.

Proposed Course: Over the 2-year period of this contract, 20 adult cancer patients will be studied in a randomized, prospective design.

Date Contract Initiated: October 1, 1976

Current Annual Level: $184,058

MEDICAL UNIVERSITY OF SOUTH CAROLINA (NO1-CP-65780)

Title: Literature Study on Indicators of Health and Nutritional Status with Emphasis on Primitive Populations

Contractor's Project Director: Dr. John R. K. Robson

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objectives of this investigation were to (1) review the methodologies used to assess health and nutritional status in primitive populations; (2) prepare a list of published and unpublished papers on the health and nutritional status of primitive populations; (3) compare the health and nutritional status of primitive peoples and populations living under Westernized, sophisticated, or industrial modes of life; (4) identify physiological, nutritional, dietary, and biochemical adaptations that might have taken place during the transition from primitive modes of living to the modern life style; (5) identify adaptations that may have relevance to carcinogenesis in modern populations; (6) identify populations that might be usefully studied in further depth; and (7) recommend protocols for such studies.

Major Findings: This contract has resulted in the compilation of a list of scientific papers relating to the assessment of nutritional status, metabolism, and health of primitive people. It also includes references to the methodology for appraising health and nutrition in primitive populations.

Significance to Biomedical Research and the Program of the Institute: Primitive populations containing subpopulations that have been partially or wholly transformed by rapid technological change and primitive populations from which subpopulations have migrated are prime research subjects for investigation of the effects of dietary and nutritional changes on human disease patterns. In particular, this literature study attempted to document the nutritional states and related health problems of primitive populations, both in their natural habitats and in transition.

Proposed Course: A workshop will be held to develop specific research ideas based on the recommendations developed under this contract.

Date Contract Initiated: June 30, 1976

Current Annual Level: $34,824 (This project was funded in FY'76 and completed in FY'77.)

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-65829)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient

Contractor's Project Director: Dr. Maurice E. Shils

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is designed to establish a relationship between the nutrition of pediatric cancer patients and the effectiveness of multi-modality therapy. More specifically, through a randomized prospective study, the project will attempt to determine whether patients provided with total parenteral nutrition demonstrate a greater tolerance for antineoplastic therapy and a better therapeutic response than similar patients receiving standard nutritional regimens.

Major Findings: This study will provide an opportunity to test the feasibility and practicality of using total parenteral nutrition to support traditional modes of anticancer therapy. It will be studied as a means of increasing survival time and improving the quality of pediatric cancer patients' lives during and after therapy. If it is found that adequate nutritional status widens therapeutic margins without proliferating tumorous growth, therapeutic regimens can be improved.

Significance to Biomedical Research and the Program of the Institute: The project is designed to assess the possible benefits of total parenteral nutrition and to answer the following questions: (1) Does optimal nutritional support enhance tumor response to radiation therapy or to various chemotherapeutic regimens? (2) Does the pediatric patient with adequate nutrition before treatment respond better to oncologic therapy? Is this response similar to that obtained in the patient who has never experienced weight loss secondary to tumor burden? (3) Can the toxic side effects of radiotherapy and chemotherapy be reduced, eliminated, or prevented by optimal nutritional support? If so, can a higher dose of therapy be administered because of reduced toxicity? Does increasing the dose result in better tumor response? If normal tissue injury does occur, does the nutritional support result in more rapid healing and decrease long-term sequelae? (4) Is tumor growth enhanced or inhibited by optimal nutritional support?

Proposed Course: Eight pediatric cancer patients undergoing similar treatment modalities for abdomen and pelvic tumors are involved in the study. Patients are assessed before, during, and after the administration of antineoplastic therapy, and their responses to therapy and nutritional support are evaluated. The experimental period (phase I) is completed within 1 year of admission to the study while the follow-up phase (phase II) lasts an additional 2 years.

Date Contract Initiated: June 30, 1976

Current Annual Level: $31,979

MIDWEST RESEARCH INSTITUTE (NO1-CP-65787)

Title: Application of Behavior Modification Techniques in the Treatment of Anorexia in the Cancer Patient

Contractor's Project Director: Dr. Sophia S. Fotopoulos

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objectives of this research are to (1) evaluate the effectiveness of behavior modification techniques in improving caloric and nutrient intake in adult and pediatric cancer patients; (2) select relevant and appropriate behavior modification techniques for the alleviation of anorexia in children and adults with carcinoma prior to, during, and after treatment; (3) measure the relationship between stages of cancer and the development of anorexia; (4) measure changes in anorexia due to the effects of cancer treatment; and (5) compare cancer patients as to their ability to eat during different clinical stages of their disease.

Major Findings: In order to improve therapy, particularly diet therapy, this project is developing behavior modification techniques to increase caloric and nutrient intake in both pediatric and adult cancer patients.

Significance to Biomedical Research and the Program of the Institute: Anorexia is common in patients with a variety of cancers. The specific factors influencing its development and progression in certain types and during different stages of cancer are not well understood.

Proposed Course: Behavior modification techniques relevant to alleviating or controlling anorexia in cancer patients have been reviewed. Specific intervention techniques will be studied in 16 pediatric and 16 adult cancer patients.

Date Contract Initiated: June 30, 1976

Current Annual Level: $138,550

NEW ENGLAND DEACONESS HOSPITAL (NO1-CP-65870)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 4--Radiotherapy, Abdomen and Pelvis Malignancies)

Contractor's Project Director: Dr. George L. Blackburn

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with abdomen and pelvis malignancies to determine its effects on tumor growth, tolerance to

antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $52,177

NORTHWESTERN UNIVERSITY (NO1-CP-65779)

Title: Anorexia in Adult and Pediatric Cancer Patients

Contractor's Project Director: Dr. William D. DeWys

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project, part of a multidisciplinary, multi-institutional cooperative effort, deals with the causes and effects of anorexia in adult cancer patients. Anorexia is considered a significant cause of morbidity in cancer patients. Researchers are collecting detailed

dietary, nutritional; anthropometric, socioeconomic, psychological, metabolic, and physiological data on 200 adult cancer patients. Both retrospective and prospective data are being collected.

Major Findings: Anorexia develops as a complication of malignancy in a significant proportion of cancer patients. In some, anorexia may be related to interference with intestinal function, while in others anorexia may be related to the endocrine or hormonal effects of a neoplasm. However, in the majority of patients, such obvious factors are not present, and anorexia is often largely unexplained. It is anticipated that the findings of these studies will help to fill this gap in knowledge.

Significance to Biomedical Research and the Program of the Institute: This effort will pave the way for future research, both of the altered metabolic processes associated with specific types of cancer and of the development of methods of increasing food intake; or perhaps it will lead to a coordinated study of the two. The project may also result in the rapid development of intervention protocols that offer immediate opportunities of supporting the traditional modes of cancer treatment (surgery, radiotherapy, and chemotherapy) and of increasing cancer survival rates.

Proposed Course: The information collected in this investigation will be analyzed to develop hypotheses on the causes of anorexia and the interrelationships between anorexia, cancer type, treatment modality, and associated phenomena.

Date Contract Initiated: June 30, 1976

Current Annual Level: in negotiation

RESEARCH FOUNDATION, STATE UNIVERSITY OF NEW YORK (NO1-CP-65834)

Title: Anorexia in Adult and Pediatric Cancer Patients

Contractor's Project Director: Dr. Giovanni Costa

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project, part of a multidisciplinary, multi-institutional cooperative effort, deals with the causes and effects of anorexia in adult cancer patients. Anorexia is considered a significant cause of morbidity in cancer patients. Researchers are collecting detailed dietary, nutritional, anthropometric, socioeconomic, psychological, metabolic, and physiological data on 300 adult cancer patients. Both retrospective and prospective data are being collected.

Major Findings: Anorexia develops as a complication of malignancy in a significant proportion of cancer patients. In some, anorexia may be related to interference with intestinal function, while in others anorexia may be related to the endocrine or hormonal effects of a neoplasm.

However, in the majority of patients, such obvious factors are not present, and anorexia is often largely unexplained. It is anticipated that the findings of these studies will help to fill this gap in knowledge.

Significance to Biomedical Research and the Program of the Institute: This effort will pave the way for future research, both of the altered metabolic processes associated with specific types of cancer and of the development of methods of increasing food intake; or perhaps it will lead to a coordinated study of the two. The project may also result in the rapid development of intervention protocols that offer immediate opportunities of supporting the traditional modes of cancer treatment (surgery, radiotherapy, and chemotherapy) and of increasing cancer survival rates.

Proposed Course: The information collected in this investigation will be analyzed to develop hypotheses on the causes of anorexia and the interrelationships between anorexia, cancer type, treatment modality, and associated phenomena.

Date Contract Initiated: June 30, 1976

Current Annual Level: $54,560

RUHL ASSOCIATES (NO1-CP-65789)

Title: Identification of Past, Ongoing and Future Dietary and Nutritional Surveys and Cancer Epidemiology Studies

Contractor's Project Director: Dr. Marjorie Whiting

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this contract is to identify completed, ongoing, and planned diet studies, nutritional surveys, and cancer epidemiology studies. The approach being used includes (1) thorough and comprehensive literature searches; (2) identification of unpublished and published studies that are not readily accessible through conventional searching resources; (3) identification of relevant foreign studies; (4) development of criteria for current and future inclusion of studies in the Diet, Nutrition and Cancer Program; (5) refinement of searching strategies and related indexing and retrieval vocabulary for incorporation into on-line bibliographic retrieval systems; and (6) development of a system for locating and monitoring ongoing research and planned studies.

Major Findings: It is anticipated that the Diet, Nutrition and Cancer Program will be able to use the results of this study to reanalyze specific hypotheses and to formulate future research efforts.

Significance to Biomedical Research and the Program of the Institute: This project was funded as preparation for intensive study on the

interrelationships between diet, nutrition, and cancer.

Proposed Course: The DNCP will evaluate and prioritize the research recommendations generated under this contract.

Date Contract Initiated: June 30, 1976

Current Annual Level: $30,643 (This project was funded in FY'76 and completed in FY'77.)

STANFORD UNIVERSITY (NO1-CP-65825)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient

Contractor's Project Directors: Dr. Sarah S. Donaldson
Dr. Jordan R. Wilbur

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is designed to establish a relationship between the nutrition of pediatric cancer patients and the effectiveness of multi-modality therapy. More specifically, through a randomized prospective study, the project will attempt to determine whether patients provided with total parenteral nutrition demonstrate a greater tolerance for antineoplastic therapy and a better therapeutic response than similar patients receiving standard nutritional regimens.

Major Findings: This study will provide an opportunity to test the feasibility and practicality of using total parenteral nutrition to support traditional modes of anticancer therapy. It will be studied as a means of increasing survival time and improving the quality of pediatric patients' lives during and after therapy. If it is found that adequate nutritional status widens therapeutic margins without proliferating tumorous growth, therapeutic regimens can be improved.

Significance to Biomedical Research and the Program of the Institute: The project is designed to assess the possible benefits of total parenteral nutrition and to answer the following questions: (1) Does optimal nutritional support enhance tumor response to radiation therapy or to various chemotherapeutic regimens? (2) Does the pediatric patient with adequate nutrition before treatment respond better to oncologic therapy? Is this response similar to that obtained in the patient who has never experienced weight loss secondary to tumor burden? (3) Can the toxic side effects of radiotherapy and chemotherapy be reduced, eliminated, or prevented by optimal nutritional support? If so, can a higher dose of therapy be administered because of reduced toxicity? Does increasing the dose result in better tumor response? If normal tissue injury does occur, does the nutritional support result in more rapid healing and decrease long-term sequelae? (4) Is tumor growth enhanced or inhibited by optimal nutritional support?

Proposed Course: Fifteen pediatric cancer patients undergoing similar treatment modalities for abdomen and pelvic tumors are, or will be, involved in the study. Patients are assessed before, during, and after the administration of antineoplastic therapy, and their responses to therapy and nutritional support are evaluated. The experimental period (phase I) is completed within 1 year of admission to the study while the follow-up period (phase II) lasts an additional 2 years.

Date Contract Initiated: June 30, 1976

Current Annual Level: $44,100

STATE UNIVERSITY OF NEW YORK AT BUFFALO (NO1-CP-65868)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 3--Radiotherapy, Squamous Cell Carcinoma of the Head and Neck)

Contractor's Project Director: Dr. John M. Lore

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with squamous cell carcinoma of the head and neck to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pretreatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function

of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $37,380

UNIVERSITY OF NORTH CAROLINA (NO1-CP-75880)

Title: Development of a Guidebook for Inclusion of Dietary and Anthropometric Parameters in Cancer Epidemiology Studies

Contractor's Project Director: Dr. Joseph C. Edozien

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The project objective is to develop guidelines for dietary and anthropometric assessment to facilitate the inclusion of these parameters in cancer epidemiology studies. Because collection and evaluation of dietary data is often subject to methodological pitfalls, this investigation will define and evaluate various methods of nutritional assessment and their limitations in relation to cancer epidemiology studies. The study will evaluate the accuracy of anthropometric instruments and develop a manual delineating essential skills for proper use of such instruments.

Major Findings: Because of its complexity, nutritional status is generally assessed through a combination of techniques. Although procedures for data collection are reasonably well known to nutritionists, their interpretation presents various difficulties. Many scientists involved in cancer research are not familiar with either specific techniques or associated problems. The development of a guidebook to describe available methods and to explain their various intents, strengths, and weaknesses will assist epidemiologists in the initial design of studies.

Significance to Biomedical Research and the Program of the Institute: The guidebook developed under this contract will be used in the training of epidemiologists and other health professionals who are involved in cancer research, prevention, and treatment. This investigation will also provide the Diet, Nutrition and Cancer Program with a basis for future analysis of methodologic developments in the assessment of the role of diet in the etiology of cancer.

Proposed Course: This investigation will incorporate the findings of these analyses into a guidebook.

Date Contract Initiated: March 1, 1977

Current Annual Level: $56,598

UNIVERSITY OF PENNSYLVANIA (NO1-CP-65791)

Title: Gustatory (Taste) Evaluation of Cancer Patients

Contractor's Project Director: Dr. Morley R. Kare

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The purpose of this study is to determine whether significant changes in taste perception occur with malignancy. Changes in taste perception have been hypothesized to be related to the anorexia of cancer patients. Specific objectives are to determine in cancer and control patients (1) basic taste preferences, (2) recognition thresholds and concentration versus intensity functions for the four taste qualities, (3) relevant taste factors affecting food preferences by means of taste panel evaluations, and (4) food preferences by survey techniques that include estimates of frequency of consumption. Results from these four components will be interpreted in relation to differences in sex, age, weight, level of activity, ratings of hunger and mood, and cancer type, extent, and treatment.

Major Findings: Fundamental information obtained from this study regarding taste changes will be utilized in formulating future research projects in behavioral and food modification regimens for anorectic patients.

Significance to Biomedical Research and the Program of the Institute: A basic knowledge of gustatory alterations in cancer patients will have immediate implications for food selection and seasoning in the production of a diet acceptable to the anorectic cancer patient.

Proposed Course: Changes in the four primary senses of taste (sweet, sour, salty, bitter) in terms of taste preference, acuity, and recognition are being examined. Taste processes are being examined before, during, and after therapy.

Date Contract Initiated: June 30, 1976

Current Annual Level: $39,882

UNIVERSITY OF PENNSYLVANIA (NO1-CP-65802)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 1--Surgery, Upper Gastrointestinal Tract)

Contractor's Project Director: Dr. James L. Mullen

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with cancer of the upper gastrointestinal tract to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $19,337

UNIVERSITY OF ROCHESTER (NO1-CP-65833)

Title: Gustatory (Taste) Evaluation of Cancer Patients

Contractor's Project Director: Dr. Dean Thompson

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The purpose of this study is to determine whether significant changes in taste perception occur with malignancy. Changes in taste perception have been hypothesized to be related to the anorexia of cancer patients. Specific objectives are to determine in cancer and control patients (1) basic taste preferences, (2) recognition thresholds and concentration versus intensity functions for the four taste qualities, (3) relevant taste factors affecting food preferences by means of taste panel evaluations, and (4) food preferences by survey techniques that include estimates of frequency of consumption. Results from these four components will be interpreted in relation to differences in sex, age, weight, level of activity, ratings of hunger and mood, and cancer type, extent, and treatment.

Major Findings: Fundamental information obtained from this study regarding taste changes will be utilized in formulating future research projects in behavioral and food modification regimens for anorectic patients.

Significance to Biomedical Research and the Program of the Institute: A basic knowledge of gustatory alterations in cancer patients will have immediate implications for food selection and seasoning in the production of a diet acceptable to the anorectic cancer patient.

Proposed Course: Changes in the four primary senses of taste (sweet, sour, salty, bitter) in terms of taste preference, acuity, and recognition are being examined. Taste processes are being examined before, during, and after therapy.

Date Contract Initiated: July 1, 1976

Current Annual Level: in negotiation

UNIVERSITY OF TENNESSEE (NO1-CP-65786)

Title: Studies of Differential Nutritional Requirements by Host and Tumor as the Basis for Dietary Treatment of Cancer

Contractor's Project Director: Dr. James T. Robertson

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this project is to evaluate the hypothesis that controlling the blood levels of a specific amino acid required by a particular glioblastoma will limit tumor growth without harming the host. The specific objectives are to (1) determine whether direct nutritional therapy alters the rate of brain tumor growth and can be used as a treatment modality; (2) determine whether direct nutritional therapy and concomitant antineoplastic therapy result in synergistic benefits; and (3) determine how direct nutritional therapy affects the host-tumor response and other associated parameters such as host-immune response. This project is a multi-institutional cooperative effort.

Major Findings: Chemotherapy and radiation therapy protocols have generally been unsuccessful in significantly prolonging the useful life of the brain cancer patient. Thus a nutritional approach to therapy, following surgical reduction of the tumor burden, is an important avenue for investigation. The project will define the feasibility of using direct nutritional therapy to treat other malignant tumor types.

Significance to Biomedical Research and the Program of the Institute: Success with this therapy modality would be an important breakthrough in the field of cancer treatment which could lead to a significant increase in the survival rates of individuals with glioblastoma tumors.

Proposed Course: Over the 2-year period of this contract, 20 adult cancer patients will be studied in a randomized, prospective design.

Date Contract Initiated: June 30, 1976

Current Annual Level: $100,000

UNIVERSITY OF TEXAS SYSTEM CANCER CENTER, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CP-65794)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Pediatric Patient

Contractor's Project Director: Dr. Jan Van Eys

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This project is designed to establish a relationship between the nutrition of pediatric cancer patients and the effectiveness of multi-modality therapy. More specifically, through a randomized prospective study, the project will attempt to determine whether patients provided with total parenteral nutrition demonstrate a greater tolerance for antineoplastic therapy and a better therapeutic response than similar patients receiving standard nutritional regimens.

Major Findings: This study will provide an opportunity to test the feasibility and practicality of using total parenteral nutrition to support traditional modes of anticancer therapy. It will be studied as a means of increasing survival time and improving the quality of pediatric cancer patients' lives during and after therapy. If it is found that adequate nutritional status widens therapeutic margins without proliferating tumorous growth, therapeutic regimens can be improved.

Significance to Biomedical Research and the Program of the Institute: The project is designed to assess the possible benefits of total parenteral nutrition and to answer the following questions: (1) Does intravenous hyperalimentation enhance tumor response to radiation therapy or to various chemotherapeutic regimens? (2) Does the pediatric patient with adequate nutrition before treatment respond better to oncologic

therapy? Is this response similar to that obtained in the patient who has never experienced weight loss secondary to tumor burden? (3) Can the toxic side effects of radiotherapy and chemotherapy be reduced, eliminated, or prevented by optimal nutritional support? If so, can a higher dose of therapy be administered because of reduced toxicity? Does increasing the dose result in better tumor response? If normal tissue injury does occur, does the nutritional support result in more rapid healing and decrease long-term sequelae? (4) Is tumor growth enhanced or inhibited by optimal nutritional support?

Proposed Course: Forty-four pediatric cancer patients undergoing similar treatment modalities for bone tumor metastases are, or will be, involved in the study. Patients are assessed before, during, and after the administration of high dose methotrexate, and their responses to therapy and nutritional support are evaluated. The experimental period (phase I) is completed within 1 year of admission to the study while the follow-up period (phase II) lasts an additional 2 years.

Date Contract Initiated: June 30, 1976

Current Annual Level: $35,096

UNIVERSITY OF TEXAS SYSTEM CANCER CENTER, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CP-65866)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 6--Chemotherapy, Testicular Carcinoma)

Contractor's Project Director: Dr. Melvin L. Samuels

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with testicular carcinoma to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion

process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $27,808

UNIVERSITY OF TEXAS SYSTEM CANCER CENTER, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CP-65871)

Title: Optimal Nutritional Support as an Adjunct to Cancer Therapy in the Adult (Segment 5--Chemotherapy, Non-Oat-Cell Carcinoma of the Lung)

Contractor's Project Director: Dr. Victor J. Lanzotti

Project Officer (NCI): Dr. Gio B. Gori

Objectives: This study is examining the use of intravenous hyperalimentation in at least 40 adult cancer patients with non-oat-cell carcinoma of the lung to determine its effects on tumor growth, tolerance to antineoplastic therapy, and host-tumor response. Using a randomized design, the study is comparing the use of routine hospital nutritional support with total parenteral nutrition in cancer patients during pre-treatment, treatment, and post-treatment periods. All of the patients being studied are on active cancer therapy regimens. Additionally, the study will determine whether specific antineoplastic therapy interferes with the efficacy of nutritional support by impairing the utilization of nutrients.

Major Findings: The data generated by this project will be a resource for future investigative efforts and will enable demonstration of the efficacy, practicality, and feasibility of adjuvant nutritional support.

Significance to Biomedical Research and the Program of the Institute: Improved techniques are now available for repleting and preventing cancer related malnutrition. The effectiveness of this method as an adjunct to the treatment of non-neoplastic cachexia and the kinetics of the repletion

process have been documented in small nonrandomized trials. The methods, however, have not been extensively applied to cancer patients. This project represents a rigorous attempt to obtain comprehensive and objective data relative to the interrelationships between total parenteral nutrition, cancer treatments, and the disease course.

Progressive cachexia is a problem during the later stages of cancer. Undernutrition is the primary cause of death in some cases of cancer and in others it is a contributory mechanism by impairing function of the liver, gastrointestinal tract, heart, skeletal muscle, and immune system. Moreover, cachexia limits the amount of radiotherapy and/or chemotherapy that patients can be given and increases surgical risks, thus hindering the effective treatment of the disease.

Proposed Course: Data collection under phase I of the study (experimental) is under way. Phase II of the study (follow-up) will be conducted over a 2-year period following the 1-year experimental phase.

Date Contract Initiated: September 30, 1976

Current Annual Level: $21,535

UNIVERSITY OF WISCONSIN (NO1-CP-65784)

Title: Survey of Dietetic Practices and Procedures used in Feeding Cancer Patients

Contractor's Project Director: Dr. Annette T. Gormican

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objectives of this project are to (1) determine the current practices of dietitians and associated health personnel in feeding cancer patients; (2) evaluate the various procedures employed; (3) report the evaluation to dietitians, clinicians, and other interested professionals; and (4) propose a series of dietary intervention protocols that could be scientifically tested and evaluated.

Major Findings: Accumulation of information concerning present dietetic practices in cancer care settings by means of a questionnaire and personal follow-up will provide guidelines for future testing of apparently successful practices. The Diet, Nutrition and Cancer Program is particularly interested in information dealing with the modification of food intake in adult and pediatric patients with anorexia. Such information is often obtained from observation by dietitians, after considerable amounts of trial and error.

Significance to Biomedical Research and the Program of the Institute: This survey is expected to uncover some uniformity in practice and to suggest some innovative dietetic approaches to dealing with the anorexia of cancer patients. It is hoped that this investigation and the dissemination of results will instigate national coordination of efforts towards

effective dietary care of cancer patients.

Proposed Course: A workshop is planned in which study results will be shared with a selected number of therapeutic dietitians working in the field of oncology.

Date Contract Initiated: June 30, 1976

Current Annual Level: $30,485 (This project was funded in FY'76 and completed in FY'77.)

Diet, Nutrition and Cancer Program Cancer Research Emphasis Grants (CREG's)

The Diet, Nutrition and Cancer Program published seven announcements in the NIH Guide on March 29, 1976 requesting applications as follows:

- Effect of carcinogens in different strains of rats
- Development of procedures for the metabolic study of adult and pediatric cancer patients
- Role of dietary fiber sources on intestinal function and cancer development in humans
- Effect of fat saturation and level on spontaneous and chemically induced cancers
- Effect of fat type and level on spontaneous and chemically induced cancers
- Epidemiologic study of dietary components and cancer development
- Effect of nutritional and environmental stress on carcinogenesis

There were 119 applications received in response to these announcements. Nine of the applications were awarded in FY'76. Current annual funding (FY'77) is $783,168. CREG's awarded by the Diet, Nutrition and Cancer Program are as follows:

CA 20908-01 R. Brown, University of Wisconsin
"Effect of Fat Saturation and Cancer Incidence in Mice"

CA 21023-01 J. Reddy, Northwestern University
"Effect of Nutritional and Environmental Stress on Carcinogenesis"

CA 20952-01 H. Sidransky, George Washington University
"Effect of Nutritional Stress on Carcinogenesis"

CA 21007-01 J. Lyon, University of Utah
"Diet and Colon Cancer in a Low Risk Population"

CA 20897-01 L. Kolonel, University of Hawaii
"Dietary Components and Cancer Development"

CA 21016-01 P. Van Soest, Cornell University
"Nutritive Quality of Dietary Fibers for Humans"

CA 20964-01 C. Blatteis, University of Tennessee
"Environmental Stress and Tumorigenesis"

CA 21008-01 J. Kinney, Columbia University
"Muscle Amino Acids and Fat Tolerance in Cancer Patients"

CA 20048-01 J. Yager, Dartmouth College
"Effect of Dietary Stress on Pancreatic Carcinogenesis"

PROJECTED PROGRAMS

At the Diet, Nutrition and Cancer Program Advisory Committee Meeting, held December 14-15, 1976, proposed projects from program definition workshops were prioritized according to the needs and resources of the DNCP. Project plans have been developed for 15 of the projects in anticipation of future funding. Following is a list of these projects:

- Development and Validation of Standard Procedures for the Nutritional Assessment and Monitoring of Adult and Pediatric Cancer Patients
- Dietary Patterns, Nutritional Assessment, and Cancer Incidence of American Vegetarians
- The Role of Nutritional Supplements in the Maintenance of Cancer Patients During Outpatient Therapy
- Study on How Nutrition and Diet are Perceived by Healthy Individuals and Cancer Patients--Implications for Cancer Therapy
- Quantification of Changes in Body Composition in Cancer Patients
- Evaluation of Pharmacologic Agents for the Treatment of Anorexia in the Cancer Patient
- In Vivo Quantification of Body Nitrogen
- Effect of Dietary Protein Type and Level on Carcinogenesis
- Evaluation of Calorie/Nitrogen Ratio of Oral Solutions Used in Feeding Cancer Patients
- Comparison of Oral Formula Feedings, Chemically-Defined Diets, and Total Parenteral Nutrition as Optimal Nutritional Support in Cancer Patients
- Nutrition Education for Physicians Who Treat Cancer Patients

- Effect of Dietary Regimens and Carcinogens in Different Strains of Rats
- Effect of Dietary Fat Saturation and Level on Carcinogenesis
- Effect of Fat Type and Level on Carcinogenesis
- Vitamin and Mineral Requirements of Cancer Patients Receiving Oral Alimentation

ALLOCATION OF FUNDS

The distribution of funds by program functional area is shown in Figure 4. Diet, Nutrition and Cancer Program expenditures for the period July 1, 1976 to September 30, 1977 are approximately $5,600,000. Of this amount, $208,296 is for a multi-institutional pediatric study supported by the Division of Cancer Treatment (NCI).

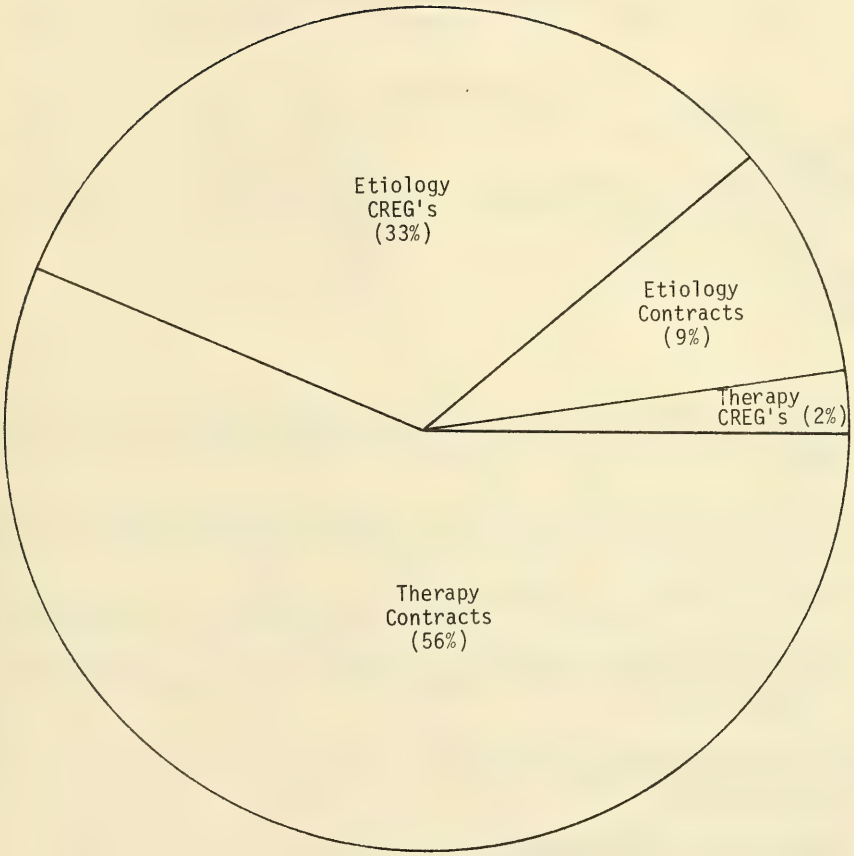

FIGURE 4. DNCP Research Budget Distribution
(July 1, 1976 - September 30, 1977)

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SUMMARY REPORT OF THE ASSOCIATE DIRECTOR
FIELD STUDIES AND STATISTICS PROGRAM
July 1, 1976 through September 30, 1977

"As life is action and passion, it is required of a man that he should share the passion and action of his time, at peril of being judged not to have lived."....Oliver Wendell Holmes, Jr.

If Holmes' standard can be applied to an organization or institution, then Field Studies and Statistics is very much alive. In the past year we have shared the passion and actions of our times adding to the action and sometimes finding ourselves surrounded by the passions.

The passion of our times is much concerned with our environment, what we are doing to it, and what it may be doing to us. The action of our times is sometimes directed toward keeping our environment from killing us slowly and insidiously many years hence, from cancer.

The staff of FS&S have contributed knowledge to lead to action in at least a dozen areas of substantial controversy in the last year--mammography, ozone radiation and skin cancer, reserpine and breast cancer, fluorides and generalized cancer, chlorination of water supplies and increased cancer, industrial hazards and cancer, asbestos, from its presence in Lake Superior water to its presence in spackling compounds, or Montgomery County rock, estrogens and endometrial and possibly breast cancer, alkylating agents and leukemia in patients treated for other cancers, TRIS and kidney cancer, saccharin and bladder cancer, chlordane and heptachlor, aldrin and dieldrin, Red #2, diethylstilbestrol, and vinyl chloride.

Our staff members have testified before Congress on saccharin, TRIS, occupations and the environment in the causation of cancer, estrogens and cancer, and, for the Subcommittee on Science and Technology, have prepared critical examinations of the CHESS program of EPA. We have served on dozens of advisory groups, ranging from the National Academy of Sciences' Committee on Safe Water to the Science Advisory Board of the National Center for Toxicological Research. Our staff have been advisors to a Swedish parliamentary commission on energy and urban pollution, to the European Clinical Cancer Research Group (Belgium), to the Japanese Research Effects of Ionizing Radiation Group (formerly ABCC), to the WHO for cancer incidence and mortality reporting, to the WHO for a reorganization of the coding of disease (ICD-O), to the American Academy of Pediatrics (Environmental Hazards), and to the NAS group investigating the risks of the chemical spill at Seveso, Italy.

If contribution to knowledge is a desired activity for a research group, and this contribution were measured by the output of scientific papers and books, then the FS&S area has been at least as active in contributing to knowledge as it has been in acting on that knowledge. The scientific staff of FS&S published, or have had accepted for publication over 200 papers in the last year. Five major books have resulted from our work:

GENETICS OF HUMAN CANCER - Mulvihill, Miller, and Fraumeni
CANCER: THE BEHAVIORAL DIMENSIONS - Cullen, Fox, and Isom
CANCER PATIENT SURVIVAL, REPORT NO. 5 - Axtell, Asire and Myers
THIRD NATIONAL CANCER SURVEY: INCIDENCE DATA - Cutler and Young
ATLAS OF CANCER MORTALITY AMONG U.S. NON-WHITES - Mason, McKay, Hoover, Blot, and Fraumeni

Ideas are the life blood of research, and the stimulation of ideas are of the most important things that a research person can do. There is no question that the publication of the two atlases of cancer mortality have been among the most stimulating books related to cancer epidemiology which have appeared in the last decade. They have led to extensive "exploratory" contracts by other government agencies, to work by various state and local governments and to much in-house research. We now have joint work in progress with several state governments, the most notable of which is New Jersey, stimulated by the maps. They have led us into exploring for (and finding) new causes for cancer into genetic, ethnic and familial studies as well as purely environmental studies.

Some of the work of the FS&S staff has been recognized in the form of awards from the Department of HEW to Dr. Fraumeni, Dr. Byar, Dr. Mason, Mr. McKay, Dr. Myers, Dr. Blot and Dr. Gail. In addition Dr. Schneiderman (along with Dr. Saffiotti, also of the Division of Cancer Cause and Prevention) received the first "Public Interest Science Award" from the Environmental Defense Fund.

There are people who believe that knowledge per se is not the great goal that some research workers make of it ("The great end of life is not knowledge, but action" -- Thomas Henry Huxley). Nonetheless, a good part of our work leads to knowledge with no clear action indicated. Thus, the observation, in a situation involving familial cancer in which three sibs and three cousins had died of acute mylegenous leukemia, a fourth sib developed the disease leading her mother to say, "I thought so, because she had the same smell as the other three," remains only an observation (i.e. knowledge) until such time as it leads laboratory workers to investigate subtle changes in smell as a possible cancer diagnostic tool. Similarly our findings of family clustering of cancer remain knowledge until other workers can take action to elucidate why the clustering. Techniques of measurement of DNA repair, of enzyme modification, of immunologic change or differences, or deficiences must be brought to bear.

Knowledge we have gained in cancer research has led to knowledge in other areas, in other diseases. In testing the immune surveillance hypothesis of cancer induction we followed over 700 military servicemen who had their spleens removed for non-illness reasons (mostly because of trauma). If the spleen contributes to immunity and immune surveillance suppresses cancer, these men should have had higher than normal cancer rates. They didn't. But they did have more deaths from pneumonia (not unexpected) and more deaths from ischemic heart disease (unexpected and hence a lead to be followed by heart disease research workers).

The FS&S area has provided help and support-as-usual to many other parts of the Cancer Institute, NIH, and other government agencies. Much of the testimony our staff gave on toxic agents was on behalf of other government agencies. We are

conducting surveys of skin cancer in 7 or 8 locations in the United States at the request of a group of Federal agencies (EPA, Department of Transportation, FDA, Consumer Products Safety Commission, etc.). Our statisticians have provided sound ways to analyze carcinogenesis studies and have laid to rest the criticisms of the NCI testing program developed from an unthinking application (by an industry statistician) of concepts of multiple significance testing. We are developing information with EPA on the effects of trihalogenated methanes (chloroform, etc.) introduced into water supplies by some water purification techniques, interacting with organic materials. Evaluation of diagnostic tests, particularly some of the immunologic measures such as CEA have been assisted by Biometry Branch members. Our involvement in the Breast Cancer Screening Program is largely, but not exclusively, in response to needs of the Division of Cancer Control and Rehabilitation. We have devised, and are carrying out randomized, controlled clinical trials of immunotherapy in lung cancer, and of more conventional therapy in breast cancer. Although enormous talent exists within FS&S in the area of controlled trials, and experiment design in general, there are still some people at NCI who do not use this reservoir of talent.

The great activity in the past year has been helped by an increase in staff, ordered by the Congress, in the Environmental Epidemiology Branch. In that Branch we have added several high level (Ph.D., M.D.) professional persons, and some technically trained professional persons at the Master's level. These are Dr. Pierre Decouflé, Dr. Charles Land, Dr. B.J. Stone, Dr. Aaron Blair, Dr. Linda Pickle, Dr. Kenneth Rothman (a visitor from Harvard University), Dr. John Boice, Dr. Kiyohiko Mabuchi, Dr. Shinkan Tokudome, Dr. Takashi Yanagawa (a visitor from Japan) and Mr. Daniel Grauman, Mr. Richard Ramsbottom, Mr. Boyd Stevenson, Ms. Patricia Hartge, Ms. Linda Morris, and Ms. Linda Mendelson. Several staff members have received advanced degrees in the past year and several persons have returned to us after assignments in other places in NCI. In other Branches, Dr. Gilbert Beebe has joined Clinical Epidemiology as an expert and Dr. Judy Bader, a pediatrician, has also joined the Clinical Epidemiology Branch.

The Biometry Branch, the largest of the three Branches, has had the greatest change in leadership with Mr. Haenszel, Dr. Chiazze and Dr. Cutler all leaving within a short time. Dr. Earl Pollack has joined the Branch from NIMH as Branch Chief.

The year has not been without trouble, problems and disappointments. The absence of a Branch Chief in Biometry and changing pressures in support of activities has been felt most acutely in the SEER program. The need to shift some support from contracts to grants has troubled many of the SEER participants, athough many of these problems are now coming under control. The most difficult and expensive part of the SEER program is the collection of followup (survival) data. Some of the SEER participants would rather spend their time and our money doing something else. A review of the followup activity is now being conducted by an outside consulting group, in an effort to develop more efficient ways of collecting these data. In addition it would seem that less use has been made of the SEER data than should be. Problems of startup, shifting staff (Iowa, Connecticut, California, Atlanta, Detroit have all had major personnel shifts or problems) have forced us to pay most of our attention to data gathering and

the quality of the data rather than to analysis. As these settle down, and with a new Branch Chief, there should be more exploitation of the SEER data.

There are several other problems that we have had and probably will continue to have. Among others there have been limited travel funds, problems of privacy, space, contracts (what is suitable for contract support and what is not?), difficulties in getting certain work started because of the (nearly) dead hand of bureaucracy, the slowness of the personnel recruiting process, and lack of understanding of the differences between in-house and grant-supported research.

TRAVEL

With an increased staff and increased responsibilities in problems of the environment (recognized by the Congress) we have had a reduced travel budget. Field studies can be conducted only in the field. Monitoring to insure high quality of data can be conducted only where the data are gathered in the field. To give us more responsibilities, and more staff to carry them out, and fewer means to do the job is schizophrenia producing. Schizophrenia should be a problem for NIMH, not NCI.

PRIVACY

Two problems exist. The first lies in the Veterans' Administration interpretation of some pre-"privacy law" legislation which has led VA to stop reporting to our SEER participants cases of cancer first seen in VA hospitals. We are under-reporting certain forms of cancer as a result. Negotiations have been conducted with the VA for the last two years, and from time-to-time it appears as if some working agreement can be reached. We are still not getting full reporting, however.

The second problem arises out of attempts to pass legislation setting rigid standards of privacy for medical records of a Catch 22 type that would, in general, make some aspects of epidemiology much more difficult, and some others impossible. We have (along with the rest of HEW) urged the Congress to refrain from any new legislation until the Department replies appropriately to the recommendations of the Commission on Privacy. At the time of this writing the entire issue was unsettled.

SPACE

Two problems: With new staff added, we have been allocated additional space in proportion to the number of new people, thus perpetuating inequities that existed before new staff was added. In addition no allowance has been made for orderly expansion of space for the additional staff mandated by the Congress in the current (FY '72) appropriation bill. Our second problem has been the contamination by toxic chemicals of a large, previously marginally usable area, devoted to our computer-related activities. Because of this contamination our computer activities have been very seriously hampered.

CONTRACTS

Some of the problems with contracts were mentioned with respect to the SEER

program. In addition we have the problem that a basic philosophy of the contract program at NCI seems to be that the contract-supported work should not be an extension of the research of the NCI staff. This strikes us as inappropriate in our field (we are not sufficiently knowledgeable in other areas to argue about the other areas) where the key to solving problems is having workable ideas, which then need to be carried out sometimes in difficult tedious detail. It seems to us that a contract program must have knowledgeable people as project officers and the only way to be effectively knowledgeable is to be actively engaged in research. Under those circumstances many contracts must be an extension of in-house research.

RED TAPE-BUREAUCRATIC BLOCKS

The major expression of the "no-loss-at-any-cost" philosophy that we have suffered from in the past year has come about in our attempts to develop skin cancer incidence data on behalf of a consortium of other federal agencies. With the major funding agency (EPA) faced with a Congressionally mandated deadline we have been delayed in starting the data gathering for almost every reason that the human mind could create ranging from whether NCI could accept EPA funds and then transfer them to NCI contractors, to whether NCI, having agreed to this data gathering because we have an existing data gathering network (SEER) would then have to go through the time consuming routine of advertising for data gathering organizations (which it already had, which is why it was asked to undertake the job). Some ways need to be developed so that work which needs to be done quickly can be done quickly. There is no evidence that any of the delays encountered in this project have improved the project, the quality or timeliness of the data, or protected the American people from inappropriate invasion of privacy.

RECRUITING

Recruiting senior people takes too long. One (acting) Branch Chief was on expert status for nine months before he was officially appointed. Even when it appears that all procedures have been scrupulously followed things come up that delay appointments. There need to be developed ways of moving more promptly.

IN-HOUSE VS GRANT SUPPORTED RESEARCH

In-house research has three characteristics that distinguish it from grant supported research. In-house research must be responsive to public and political needs. No grant-supported research worker is required to stop what he is doing in order (for example) to carry out a study on the putative hazards of fluoridation. No grant-supported research worker is labelled a murderer in the CONGRESSIONAL RECORD if his findings don't support some highly placed person's prior belief. Second, the in-house worker can undertake work which may take years before a culmination comes about. The grant-supported worker, because of the system of funding on short-term renewable grants, cannot afford to have no tangible products for any appreciable time. Finally, no grant-supported worker can take the risk of working on a problem that might have no payoff. The in-house worker, because of his continuing support can take this risk even though it might be potentially damaging to his

career within his discipline. The net result of these differences has been that in epidemiology, at least the best work has come from people with long-term guaranteed support. In the United States this has been from people in the Federal and State Governments, or people who are part of on-going institutions such as the American Cancer Society, and those few academic workers who have been supported by program project grants. In Great Britain the equivalent situation exists, with MRC (Medical Research Council) units with a substantial guarantee of continuity doing the recognized best work. The differences in how useful research is conducted in epidemiology in contrast to the short-term laboratory experimentation need to be recognized in funding and review mechanisms. Ways need to be found to fund academic epidemiologists for long-term and sometimes non-revealing studies, without damage to their careers and their further funding.

DISAPPOINTMENTS

There have been some disappointments in the past year leavening our substantial successes. We were disappointed that the controlled trial of the 13-retinoids for the prevention of lung cancer could not be carried out among the asbestos workers in Tyler, Texas. Our associations with the Soviet epidemiologists have not been as fruitful as we had hoped. The planned joint volume is still a planned joint volume. As of this date (June 1977) the Soviets have submitted nothing to meet the agreed-upon October 1976 submission date. The CREG program has not produced as many good, viable projects as we had expected. Our cooperative efforts with NIOSH on occupational cancer still do not involve as close cooperation and joint project management as we think most conducive to useful outcomes. (We may become more closely involved in the next year because of NIOSH's need to make up for its loss of several of its epidemiologists.) We have been distressed at the proliferation of patient data and cancer reporting systems but recent action bringing several groups together gives promise of working out this problem.

PLANS AND PROSPECTS

Our plans for next year involve exploitation of leads on hand, substantial cooperation with other government agencies, and development of ways to assess the progress of other aspects of the cancer program. Specifically, we will do more work on high risk areas, involving as many of the state and local government people as possible. SEER will be used as a base for case-control studies, and from SEER data we expect to develop new indexes of progress of the cancer program, such as site standardized survival patterns, reviews of technology transfer (in treatment), the indicators for optimal treatments, etc. We will do work on the carcinogenic potential of drugs, including those used to treat cancer and will expand our work on the problems of contaminants in water, including new systems for sampling water for contaminants. We plan to do more work on industrial hazards. We will work on problems of personal and familial susceptibilities, welding even more closely the host and environmental interactions. In our research on problems of nutrition as causes of cancer, we plan to follow up the work of Haenszel, Berg and Correa, merging knowledge in epidemiology and pathology.

We plan to develop new methodologies for extrapolation from animal to man,

particularly in the case of multiple exposures, and special short-term exposures, such as breast feeding when the mothers' milk contains carcinogens. We will develop experiment designs to allow for the economical testing of combinations of agents. We will develop sampling techniques to permit the more efficient use of the "scarcity professions" such as veterinary pathologists in carcinogenesis testing.

We will continue to encourage etiologic studies of childrens' cancers, including more extended family studies. We will support (through professional input) studies of psychological factors, associated with cancer and cancer treatment (e.g. mastectomy). By the end of the year the data collection ends file maintenance of the Veterinary Medical Data Program will be turned over to a cooperative of VMDP universities.

We also anticipate (and in this sense have planned for) doing a great many things we have not planned for. The problem-of-the-year of 1978 is not likely to be saccharin, PCB's, chloroform, fluorides, mammography, TRIS, or anything else we worked on this year .

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04405-03-FSS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Bender-Gestalt Perceptual-Motor Test Studies in Cancer and Other Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	: William Regelson	Chman., Dept. of Oncology	Med. Coll. of Va.
OTHER:	Bernard H. Fox	Manager, Social Science	FSS, NCI
	Phyllis A. Hornbuckle	Asst. Prof., Psychology	Med. Coll. of Va.
	J. Robert Butler	Prof., Psychology	North Texas Univ.

COOPERATING UNITS (if any)

Medical College of Virginia
North Texas University

LAB/BRANCH

Office of the Associate Director, Field Studies & Statistics Program, DCCP

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.18	.15	.03

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

In a study of palliation of cancer treatment effects, Bender-Gestalt test results given routinely showed remarkably high average error totals when compared with results found in a college standardizing population. Age-corrected analysis of these results suggested that age contributed to the high average. To check on the possibility that the Bender could detect neurological deficits not clearly visible in normal behavior, 125 patients were tested before biopsy on the Bender, the WAIS digit-symbol subtest, and a covert and overt anxiety test. Results of two groups were compared: 25 patients whose biopsies disclosed cancer and 25 age-, sex-, and race-matched non-cancer patients with matching on organ site where possible. Neither Bender nor digit-symbol showed substantial differences, even when adjusted for covariance of either anxiety mode. The control group showed greater anxiety in both modes than the cancer group, significantly so ($p < .01$) for covert anxiety. Repression of anxiety, hypothesized elsewhere, was tentatively disconfirmed. Since the Bender scoring method was new, standard scoring will be carried out and the data reanalyzed.

PHS-6040
(Rev. 10-76)

Project No.: Z01-CP-04405-03-FSS

Project Description

Objectives:

To investigate the ability of the Bender-Gestalt perceptual-motor test, when given before biopsy, to distinguish cancer and non-cancer patients.

Methods Employed:

One hundred twenty-seven patients were tested before biopsy on the Bender-Gestalt test, two anxiety measures, and the digit-symbol subtest of the WAIS, a standard intelligence test. Results of two groups were compared: 25 patients whose biopsies disclosed cancer and 25 age-, sex-, and race-matched non-cancer patients, with matching on organ site where possible. A modified Bender scoring system was used.

Major Findings:

The matching process (especially age) eliminated the substantial differences in Bender results found earlier in a comparison of unmatched cases and controls, although the cases showed a small excess of errors over the controls. Digit-symbol scores in the two groups were about the same. Statistical adjustment for anxiety (covariance) had little effect on the small differences found in the Bender or the digit-symbol results. The control group showed more anxiety than the cancer group, significantly so for covert anxiety ($p < .01$). However, Bender scores were standardized on a college population, hence are subject to question.

Significance to Biomedical Research and the Program of the Institute:

Failure to discriminate the groups tested shows that a substantial perceptual-motor deficit as measured by the Bender using this scoring system or the digit-symbol test is not characteristic of the cross-section of cancer patients at the stages seen here. We have not eliminated these possibilities: that poor perceptual-motor performance may be characteristic of patients with cancer at certain specific sites; that the small deficit found, though not significant, is nevertheless real; or that deficits are characteristic of hospitalized pre-biopsy patients, whether cancerous or not. Lower covert anxiety in the cancer cases is contrary to the usual repression hypothesis. A socio-economic difference, not explored here, could yield such results.

Proposed Course of Project:

Unsuccessful efforts were made to find age norms in general and to find the original norms on the modified scoring system. Because of this failure, the Bender protocols will be rescored using a standard system and reanalyzed.

Date Initiated:

May, 1974

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04406-03-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Identification of Cancer Patients by Neuromuscular Evidences in Handwriting

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	: Samuel Litwin	Chief, Statistics Div.	Inst. for Cancer Research
OTHER:	Bernard H. Fox	Manager, Social Science	FSS, NCI
	Daniel G. Miller	Director	Strang Clinic
	Irene Blaser	Research Associate	Strang Clinic

COOPERATING UNITS (if any)
Institute for Cancer Research, Philadelphia, Pa.
Strang Clinic, New York, N.Y.

LAB/BRANCH
Office of the Associate Director, Field Studies & Statistics Program, DCCP

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.15	.10	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A number of reports have appeared, most by one person, that purport to show an ability to identify cancer patients by specific handwriting characteristics at a microscopic level, presumably evidence of neuromuscular dysfunction associated with cancer. The claimed power of discrimination was very high. Some questions about the possibility of non-blind experimentation left a cloud over the validity of the reported findings. This study was intended to test the claim with rigorous, blind analysis of handwriting samples, code-breaking done by persons other than the writing analysts, and between- and within- analyst reliability trials. The finding of 60% and 58% correct overall for 50 cancer and 50 non-cancer specimens were significantly different from chance ($p < .05$) when χ^2's for the two readers were added. However, the unreliability, both within and between readers, tended to nullify a conclusion of successful discrimination. Further, on a repeat trial with cooperative reading, agreement was reached in 72 out of 80 readings. The number correct (39) was not significantly different from chance. It is concluded that handwriting of cancer and non-cancer patients cannot be distinguished reliably in a general screening situation.

PHS-6040
(Rev. 10-76)

Project No.: Z01-CP-04406-03-FSS

Project Description

Objectives:

To determine whether cancer patients can be identified by evidences of neuromuscular dysfunction in handwriting.

Methods Employed:

Handwriting samples of 50 cancer and 50 non-cancer patients were analyzed microscopically on six criteria in a study using blind design, between- and within-reader reliabilities, and cooperative reading trials.

Major Findings:

In the initial study, 60% and 58% of the judgments about presence of cancer were correct ($p < .05$ for combined χ^2's of readers). However, reliability, both within and between readers, was so poor as to nullify a conclusion of successful discrimination. On a repeat trial with cooperative reading, agreement was reached in 72 out of 80 readings. The number correct (39) was not significantly different from chance expectation. It is concluded that handwriting of cancer and non-cancer patients cannot be distinguished reliably in a general screening situation.

Significance to Biomedical Research and the Program of the Institute:

Identification of cancer patients at a success level close to that found in earlier reports was hoped for. Such a success level would form the basis for studies in which attempts would be made to identify presymptomatic cancer victims in a screening process. The result of this study, combined with those of an earlier unsuccessful attempt, also well controlled, lays to rest that hope, and will divert research efforts away from an area with no apparent payoff.

Proposed Course of Project:

No further direct effort is envisaged, although the investigator will provide very limited consultation to organizations that may decide to carry forward the inquiry with site-specific studies, an effort that has more chance of showing payoff if it exists at all. Papers for presentation and publication are being written.

Date Initiated:

October, 1974

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04407-03-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Evaluation of Single and Double Cervical Smears

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bernard H. Fox Manager, Social Science FSS, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Office of the Associate Director, Field Studies & Statistics Program, DCCP

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.02	.02	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A model of the rates of discovery of positives and true and false negatives for the cases of single and double screening determinations (especially cervical cancer) was developed for a special case. In this model positives were defined as screening outcomes resulting in some further action involving further cost--diagnostic or treatment--whether justified by later verification or not. For Pap smears the literature shows a substantial improvement in number of positives discovered by using double smears. Using this model, prevalence of positives can be estimated with one screening using double smears on a given cohort and prevalence and incidence in two screenings. Sources of data to estimate benefit/cost improvements by use of double smears are being explored.

PHS-6040
(Rev. 10-76)

Project No.: Z01-CP-04407-03-FSS

Project Description

Objectives:

To analyze the possible improvement or reduction in benefits associated with double screening determinations for the case where a positive finding is specially defined.

Methods Employed:

The first phase of a screening process is modeled, where positive findings result in further investment--extension of diagnostic procedures or treatment. The results of single and double screening determinations are examined in the model. Cost estimates will be made on the basis of actual clinic experience and relative costs of double and single determinations will be compared.

Major Findings:

Preliminary calculations show an important improvement in discovery rates of moderate dysplasias or worse, but point to a less dramatic improvement in discovery rate of cancers in-situ or worse.

Significance to Biomedical Research and the Program of the Institute:

It is expected that costs for double smears will not increase over those for single in proportion to the increased discovery rates for moderate dysplasia or worse. Because identification of moderate dysplasia is less reliable than that for severe dysplasia or worse, the improvement in discovery rate per unit cost for the latter may be somewhat less than for moderate dysplasia. Nevertheless, it is expected to be substantial. If so, such a finding would indicate the desirability of a widespread change in cervical procedures.

Proposed Course of Project:

The model, at present too diffuse, will be rewritten to focus on the central issue. A joint effort will be sought with DCCR, and an RFP to examine costs and other aspects of doing double smears will be proposed and submitted.

Date Initiated:

October, 1974

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04413-02-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Suicide Among Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Bernard H. Fox	Manager, Social Science	FSS, NCI
OTHER:	Max H. Myers	Director, SEER Program	FSS, NCI
	Suellyn Boyd	Staff Fellow	FSS, NCI
	John T. Flannery	Director	Connecticut Tumor Registry

COOPERATING UNITS (if any)
Connecticut Tumor Registry, Connecticut State Department of Health

LAB/BRANCH
Office of the Associate Director, Field Studies & Statistics Program, DCCP

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.30	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Although it is well known that some cancer patients commit suicide because of the disease, data on the suicide rate among cancer patients are very soft, only a few reports appearing, with small numbers. It is suspected that the age-adjusted rate is higher than that for the population. Comparisons with rates of patients having other illnesses are mostly anecdotal or not age corrected. It is proposed, in this study, to determine suicide rates among cancer patients from cause-of-death reports in a cohort of patients from one or more SEER cancer registries over several years. Age-adjusted cohort rates will be compared with population rates. If enough cases are available, the relationship of cancer site and suicide rate will be analyzed. Although only limited data are available for other diseases, rates for cancer patients and those with other diseases will be compared.

PHS-6040
(Rev. 10-76)

Project No.: Z01-CP-04413-02-FSS

Project Description

Objectives:

To determine suicide rates among cancer patients in general and for particular sites, and to compare these rates with population rates.

Methods Employed:

SEER cancer registries will form the data source, and standard techniques will be used.

Major Findings:

Significance to Biomedical Research and the Program of the Institute:

Since existing information on suicide rates in cancer patients is very soft (few studies with few cases), the existence of SEER registries permits a broad analysis with hard data. Further since available evidence points to rates higher than those for the age-equivalent population, we have a clear case where competing risks are not independent. With availability of hard data, different models of dependent competing risks could be tested directly, and better estimates of adjusted cancer survival probabilities could be made.

Proposed Course of Project:

A preliminary run on the Connecticut Registry for overall statistics has been carried out for detailed planning purposes. An analytic plan has been set up and data extraction is proceeding. The Connecticut analyses should be finished in the fall.

Date Initiated:

April, 1976

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04414-02-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Relation of Premorbid Psychological Factors to Cancer Incidence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bernard H. Fox Manager, Social Science FSS, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Office of the Associate Director, Field Studies & Statistics Program, DCCP

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.15	.10	.05

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The association of premorbid psychological factors--stress, personality, social environment--and cancer incidence has often been reported as present and occasionally as absent. Reviews of such studies, however, have pointed to possible flaws in their experimental design, with resulting questions about their conclusions. In addition, almost none of the studies has taken into account certain factors having an important impact on outcomes. In these studies, each member of a cohort, presumed initially without cancer, will have produced data on psychological factors on entry. The cohorts will be followed to a point where enough cases of cancer have occurred to detect an excess or deficient risk of cancer in association with specific patterns of psychological factors. When possible, predictions will be made in advance on the basis of prior hypotheses.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04414-02-FSS

Project Description

Objectives:

To determine if premorbid psychological factors--stress, personality, social environment--are associated with incidence of cancer.

Methods Employed:

A presumed cancer-free cohort will be chosen, psychological factors will be measured or will have been measured for each person on entry into the study and, if possible, during its progress. Cancer incidence will be observed over several years. Hypotheses relating certain patterns of premorbid psychological factors and probability of getting cancer will be tested.

Major Findings:

Significance to Biomedical Research and the Program of the Institute:

Repeatedly NCI has received questions from professionals and the public about the relation among psychological factors, stress and cancer incidence. These questions were based on a long history of belief, speculation and purported evidence that stress and certain psychological factors increase cancer risk. Evidence on both humans and animals, though voluminous, is not persuasive, and in some cases, contradictory. Most of the human studies have not taken into account certain factors having an important impact on outcomes. Studies with improved design are, therefore, much needed, and this project fulfils that need, in part. A positive answer would give impetus to studies on specific psychological factors or stress and the humoral environment associated with increased cancer risk. A negative answer would tend to shift the balance of research effort away from the psychic components of environmental carcinogenesis, whether internal or external to the body.

Proposed Course of Project:

A CREG announcement has been issued. Grant applications were received with a deadline of February 1, 1977. The normal course of awards will follow, with Study Section meeting in late July, 1977 and Council in October, 1977. The projects themselves are expected to last five years. An extensive pre-application paper was written as information for prospective applicants. More than 400 requests for this were received. It will be revised and published.

Date Initiated:

October, 1975

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04475-01-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Special Nonmelanoma Skin Cancer Incidence Survey and Epidemiology Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Scotto	Senior Hlth Service Officer	FSS,NCI
Other:	Thomas R. Fears	Mathematical Statistician	FSS,NCI
	Gio B. Gori	Deputy Director	DCCP,NCI

COOPERATING UNITS (if any)
Herbert L. Wiser, Ph.D., BACER Program, Environmental Protection Agency

LAB/BRANCH
Office of Associate Director, Field Studies and Statistics Program,DCCP

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health,Bethesda,Md.,20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	-

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to provide more data relative to skin cancer and harmful solar ultraviolet. An urgent need for these data has existed since recent scientific reports have warned about the decomposition of stratospheric ozone by nitrogen oxides and chloroflouromethanes (CFM's). Federal regulatory agencies have already recommended bans on the use of aerosol spray cans which use CFM's as propellants. In the near future, there will be critical reviews of all pertinent information by government as well as individual interests concerned with the threat of increased human skin cancer due to excess amounts of UV-B reaching the earth's surface, as its protective ozone shield is depleted.

PHS-6040
(Rev. 10-76)

Project ZO1-CP-04475-01-FSS

Project Description

Objectives:

The objectives of this project is to provide data relative to skin cancer and harmful ultraviolet radiation. In particular: (1) To conduct UV-B measurements at different United States locations; (2) To determine the incidence of nonmelanoma skin cancer in various population groups within the United States; and (3) To ascertain and measure epidemiological factors which may contribute toward the excess risk of nonmelanoma skin cancer in specific population groups.

Methods Employed:

Using Robertson-Berger meters, UV measurements were obtained at ten locations in the United States during 1974. These data were presented in detail and should be useful to those who need to restrict their sunlight exposure. The available nonmelanoma skin cancer data were compared with the UV-data.

Major Findings:

We estimate that each 1% decrease in ozone will result in a 2% increase in UV-B reaching the surface of the earth which in turn will result in a 4% increase in skin cancer among White Caucasians in the United States.

Significance to Biomedical Research and the Program of the Institute:

These basic epidemiologic studies will reduce the degree of uncertainty of the dose-response relationship of ozone depletion and increased skin cancer induction.

Proposed Course of Project:

An interfederal agency task force, the BACER (Biological and Climatic Effects Research) program, provided $840,000 as pass through funds from its lead agency, the EPA (Environmental Protection Agency), to the NCI for the purpose of conducting epidemiologic skin cancer surveys in as many of NCI's SEER Program locations as possible. Under this cooperative interagency agreement between the EPA and the NCI, the NCI for its part will use EPA funds to partially support the supervision and conduct of a special morbidity survey as follows: 1) Nonmelanoma incidence studies at several SEER locations to supplement four of the earlier TNCS sites; 2) Pilot study on nonmelanoma patients in TNCS study in Minneapolis to determine exposure habits, skin types, etc.;3) If pilot study indicates that it is appropriate then conduct case/control studies to determine ethnic background, skin pigmentation, and exposure habits of skin cancer patients and controls.

Proposed Course of Project (continued)

Seven SEER locations have expressed their willingness to participate in this study (Seattle, San Francisco-Oakland, Utah, New Mexico, New Orleans, Atlanta and Detroit). We expect the study to begin in these locations by June 1, 1977.

Date Initiated:

1974

Publications:

Articles Published in a Periodical:

1. Fears, T.R., Scotto, J., and Schneiderman, M.A.: Skin cancer, melanoma, and sunlight. AJPH 66: 461-164, 1976.

2. Fears, T.R., Scotto, J., and Schneiderman, M.A.: A Comment on "Skin cancer, melanoma and sunlight". AJPH 67: 59-60, 1977.

3. Scotto,J., Fears, T.R., Gori, G.B.: Ultraviolet exposure patterns. Environmental Research. In press.

4. Scotto, J., Fears, T.R.: Intensity patterns of solar ultraviolet radiation. Environmental Research. In press.

5. Fears, T.R., Scotto, J., and Schneiderman, M.A.: Mathematical models of age and ultraviolet effects on the incidence of skin cancer among whites in the United States. Am. J. of Epid. In press.

Articles in a Book, Pamphlet, or Bulletin:

1. Scotto, J. and Fears, T.R.: Skin Cancer in the United States. Schneiderman, J.A. and Levin, D.L. (Eds.): Monog. Cancer in the USA and USRR. To appear.

2. Scotto, J. and Fears, T.R.: Skin Cancer Epidemiology-Research Needs. In: Proceedings of the International Conference on Ultraviolet Carcinogenesis. To appear.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04476-01-FSS

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cancer Mortality and Asbestos Deposits

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas R. Fears, Ph.D. Mathematical Statistician FSS,NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Associate Director,Field Studies and Statistics Program, DCCP

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Md.,20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Asbestos deposits are found in many parts of the United States. In their work we asked: Is there an increase in risk from cancer associated with naturally occurring asbestos?

PHS-6040
(Rev. 10-76)

Project No.Z01-CP-04476-01-FSS

Project Description

Objectives:

To determine the magnitude of the cancer risk associated with naturally occurring asbestos deposits.

Methods Employed:

In an attempt to control for the urban effect, geographic gradient and socioeconomic class, each county in the United States with asbestos deposits was matched for percent of area that was urban and for median years in school with two nearby counties that did not have known asbestos deposits.

Major Findings:

No significant differences for asbestos county mortality rates compared with control county mortality rates were found for males or for females.

Significance to Biomedical Research and the Program of the Institute:

The study of cancer mortality rates in these matched counties provides no evidence that naturally-occurring asbestos is a great hazard to the general population of counties with asbestos deposits.

Proposed Course of Project:

-

Date Initiated:

May, 1976

Publications:

Articles Published in a Periodical:
Fears T.R.: Cancer mortality and asbestos deposits. Am.J. of Epidemiology 104: 523-526, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04477-01-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Procedures for Reducing the Pathology Workload in the Carcinogen Bioassay Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas R. Fears	Mathematical Statistician	FSS,NCI
Other:	J. Fielding Douglas	Expert	CBPR,NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Associate Director,Field Studies and Statistics Program, DCCP

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md., 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Large test programs have been set up to help assess the carcinogenic potential of chemicals introduced in the environment. Most test protocols used in these programs require large numbers of animals and extensive pathologic evaluation of all test and control animals. This project was initiated to find modifications to the present protocol which will reduce the pathology workload of the program and make only small changes in the operational character of the screen.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

We propose changes in the NCI experimental protocol which would reduce the pathology workload by requiring examination of a "reasonably large number" of animals rather than all animals.

Methods Employed:

The first modification derives from the fact that a tumorigenic effect may be ruled out by looking at the treated animals only. The second procedure suggested uses simple random samples to provide information about the treated groups with still less pathology work.

Major Findings:

Using data from experiments conducted under NCI contract we computed typical savings under each procedure. Results suggest that implementing these procedures would provide a large reduction in pathology workload.

Significance to Biomedical Research and the Program of the Institute:

By reducing the pathology required to test a compound, we obtain a large reduction in the cost of testing a chemical.

Proposed Course of Project:

Since these pathology protocols have not been put into use, they will be used in several experiments in parallel with traditional protocols to help assess their feasibility and accuracy. The modifications proposed should be regarded as a starting point for discussion. We anticipate many comments from interested scientists.

Date Initiated:

May, 1976

Publications:

Articles Published in a Periodical:

Fears, T.R., Douglas,J.F.: Suggested Procedures for Reducing the Pathology Workload in the Carcinogen Bioassay Program. J.of Environmental Pathology and Toxicology. To appear.

Fears, T.R., Douglas,J.F.: Suggested Procedures for Reducing the Pathology Workload in the Carcinogen Bioassay Program II: Incorporating Blind Pathology Techniques and Analysis for Animals with Tumors. J.of Environmental Pathology and Toxicology. To appear.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04478-01-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
False Positive and False Negative Rates for Carcinogenicity Screens.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas R. Fears	Mathematical Statistician	FSS,NCI
	Robert E. Tarone	Mathematical Statistician	FSS,NCI
	Kenneth C. Chu	Asst.Manager/Data Analyst	CBPR,NCI
Other:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Associate Director,Field Studies and Statistics Program,DCCP

SL

INSTITUTE AND LOCATION
National Cancer Institute,National Institutes of Health,Bethesda,Md.,20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	-

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The implementation of a number of chemical carcinogen screening programs has been accompanied by the observation that some screens might have high false positive error rates. Bounds on the false positive and false negative error rates of the NCI screen have been computed.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To determine bounds on the false positive and false negative error rates of the NCI carcinogen screen.

Methods Employed:

Appropriate standard calculation.

Major Findings:

With designs presently used at the National Cancer Institute and historical spontaneous tumor rates based upon control animals in previous experiments, we compute upper bounds on the false-positive error rates for several screening strategies. False-positive results are much less likely to occur at tissue sites with low spontaneous tumor rates; hence the site at which a significant tumor increase occurs is important. There is danger in relying solely upon the finding of statistical significance without incorporating biological knowledge and corroborative evidence such as the presence of a dose-response relationship or experimentally consistent results in different species or sexes.

Significance to Biomedical Research and the Program of the Institute:

We have established that the false-positive error rate of the NCI carcinogen screen is small.

Proposed Course of Project:

-

Date Initiated:

June, 1976

Publications:

Articles Published in a Periodical:

Fears, T.R., Tarone, R.E., and Chu, K.C.: False positive and false negative rates for carcinogenicity screens. Cancer Research. To appear.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04482-01-FSS

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Psychological and Other Predictors of Remission Time After Surgery in Clinical Stage II Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Bernard H. Fox	Manager, Social Science	FSS, NCI
OTHER:	Suellyn Boyd	Staff Fellow	FSS, NCI
	John Horm	Statistician	FSS, NCI
	Larry R. Muenz	Statistician	FSS, NCI
	G. Nicholas Rogentine, Jr.	Senior Investigator	I, NCI
	Richard Haier	Psychologist	LPP, NIMH

COOPERATING UNITS (if any)
This is a sub-project of a broader one, Project Z01-CB-05052-02-I, Psychosocial Factors in Prognosis of Melanoma, whose PI is G. Nicholas Rogentine, Jr.

LAB/BRANCH
Office of the Associate Director, Field Studies & Statistics Program, DCCP

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.47	.45	.02

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS (Human tissues examined in main project)

SUMMARY OF WORK (200 words or less - underline k

In this study an attempt is made to quantify the ability of psychological and other tests to predict time to relapse or remission status at given intervals, among stage II melanoma patients after axillary node surgery. Initially discrimination of relapse before and after one year was found for subjective effect of having melanoma on life style after controlling for blood type and number of nodes. All three were weighted about equally in a discriminant analysis. Certain MMPI results were successful predictors when examined alone. With increase in number of cases and with approaches capable of much less biassed testing of post-hoc hypotheses than basic discriminant analysis, new estimates of predictive capability will be made.

PHS-6040
(Rev. 10-76)

Project No.: Z01-CP-04482-01-FSS

Project Description

Objectives:

To test hypotheses that certain psychological measures will predict duration of remission after controlling for known biological predictors.

Methods Employed:

Variables were used that had singly discriminated relapsers and remitters at one year: number of nodes, blood type and subjective effect of having melanoma. A multiple regression was done on relapsers only. Discriminant analysis was done for one year status of relapse or remission. Separate analyses of MMPI results were attempted, both as individual variables and as multiple, with and without biological predictors. Further analysis will be done as new cases come in and as remission times are extended. Approaches will be used capable of less biassed testing of post-hoc statistical hypotheses than the above tests.

Major Findings:

For relapsers before one year only the variable "number of nodes" was related significantly to number of days remission in a multiple regression. In the discriminant analysis the three variables had about equal weights, all significant, and predicted 29 out of 31 cases correctly. Some MMPI variables were significant when taken alone, especially number of scales above 70 and the lie scale. When taken with the three other predictors MMPI predictive capability dropped to low levels. These findings are preliminary, but point to the value of analyzing newly emerging data.

Significance to Biomedical Research and the Program of the Institute:

If psychological variables are found to have independent weight in predicting remission time, their relationship with some physical attributes should be sought, to establish why they are predictors. The most important outcome would entail a cause-effect relationship of psychic state and such physical variables, thus permitting change and possible extension of remission. Even without such a relationship, a predictive capability of psychological measures would be valuable to the clinician, and under some conditions, perhaps to the family and the patient.

Proposed Course of Project:

As the sample grows we will have more confidence in our estimates of the ability of psychological variables to predict remission time. A sample of about 70 is expected by the end of FY 1977, with a final sample of about 120 in a year. 18 months after that, enough time should have gone by to produce good estimates of weights in a predictive equation rather than the very tentative ones we have now.

Project No.: Z01-CP-04482-01-FSS

Date Initiated:

August, 1976

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE DIRECTOR
FIELD STUDIES AND STATISTICS PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION

FISCAL YEAR 1977

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-43342)

Title: Etiology of Esophageal Cancer in the Caspian Littoral of Iran

Contractor's Project Director: Dr. Calum S. Muir

Project Officer (NCI): Dr. Marvin A. Schneiderman

Objectives: To determine the causes of the high rate of cancer of the esophagus in the Caspian Littoral of Iran and to explain the variation in rates from place-to-place--the eventual purpose being to develop an understanding of mechanisms of the increase in cancer of the esophagus in the black population in the United States, which may then lead to appropriate intervention that would stop this increase, and possibly reduce the incidence. To do this involves case-control studies with emphasis on diet and nutritional status, food contaminants, use of possibly harmful substances (e.g. sukhteh), and laboratory studies of susceptibility factors, immunogenetics, enzyme activity, and finally studies of the pathology of the esophagus in both humans and animals in the high and low incidence areas.

Major Findings:
1. A strongly mutagenic agent has been found in an opium product which is commonly eaten in the high incidence areas.
2. Wheat and bread samples have not revealed any unusual biological contaminants (e.g. nitrosamines, aflatoxins, other mycotoxins).
3. A high prevalence of riboflavin deficiency and possible vitamin A deficiency has been found.
4. The disease is highly associated with a Turkic-Mongol genetic profile.
5. A detailed hypothesis of the mechanisms of the etiology of the disease has been elaborated which should serve as a general unifying umbrella covering all the studies of etiology of cancer of the esophagus around the world.

Significance to Biomedical Research and the Program of the Institute:
Cancer of the esophagus is increasing in blacks (and not in whites) in the United States. The usual explanations (alcohol and tobacco) seem to be defective. This problem needs to be worked out. The hypothesis of mechanism of cancer of the esophagus developed by the IARC group shows promise of moving us toward appropriate explanations. The interactions with high quality laboratory workers, involved in generalizable biological research has pulled together genetic and environmental factors in a way that could open out this whole problem to solution. Data collection and detailed statistical analyses of epidemiological studies are almost always long-term. It is anticipated that these studies will provide information on the etiology of cancer of the esophagus (which is increasing rapidly in the black population of the United States) that can be used to prevent this cancer. The interactive nature of the carcinogenic

process needs to be further elucidated. The increase in esophageal cancer in blacks in the United States follows the large migrations from rural to urban areas. In whites, cancer of the esophagus has been largely an urban disease. Aspects of urban life that increase exposure or susceptibility need to be investigated.

Proposed Course: To test the umbrella hypothesis developed during the past two years the following work will be done:

1. Comparison (case-control) of households with respect to:
    a. diet and nutritional status
    b. use of opium products
    c. exposure to other contaminants
    d. genetic and immunologic markers
    e. enzymatic measures

2. Further laboratory characterization of wheat products, opium products, and other possible mutagens.

3. Measures of esophageal function:
    a. mobility
    b. precancerous lesions
    c. capacity of esophageal tissue for DNA repair

It is estimated that adequate information should be developed within two years to elucidate these issues sufficiently to decide whether:

1. No further useful work can be done in Iran on this problem, or

2. Enough was known that intervention measures should now be taken.

Date Contract Initiated: January 1, 1973

Current Annual Level: $100,000

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE DIRECTOR
FIELD STUDIES AND STATISTICS PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

UNIVERSITY OF CALIFORNIA, LOS ANGELES (NO1-CP-33327)

Title: Group Testing for Screening Carcinogens

Contractor's Project Director: Dr. Robert M. Elashoff

Project Officer (NCI): Dr. Thomas R. Fears

Objectives: To develop mathematical techniques to permit more rapid and efficient testing of chemicals for potential carcinogenesis.

Major Findings: During the last year all the data sets for the mixture experiments have been obtained, have been corrected and edited. All the computer programs to carry out the data analysis have been written, debugged and tested. The statistical results now obtained provide a basis upon which the animal carcinogenicity testing program can be carried out in a more economical way than in the current program, without sacrificing either the sensitivity of specificity.

Significance to Biomedical Research and the Program of the Institute: Carcinogenesis testing is a long-term expensive process. With over 20,000 materials to test for carcinogenicity unless more efficient methods are developed testing will take a very long time. This research holds promise for more rapid testing.

Proposed Course: Continued close collaboration with groups doing biological testing of the materials to speed the development of mathematical models.

Complete development of one at a time two stage testing scheme.

Complete analysis of long-term experiments.

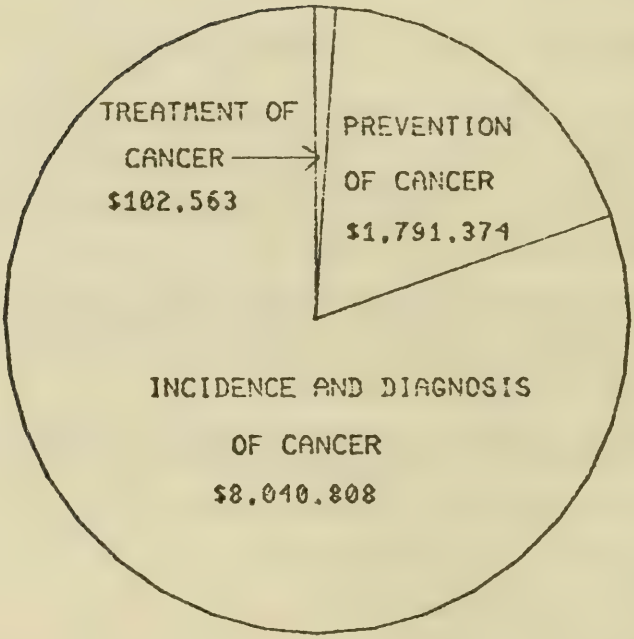
Date Contract Initiated: June 24, 1971

Current Annual Level: $92,610

FIELD STUDIES AND STATISTICS PROGRAM

DISTRIBUTION OF CONTRACT FUNDS

Fiscal Year 1977



Total Contract Funds: $9,934,745

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES

FIELD STUDIES AND STATISTICS

FY 1977

TYPE OF ACTIVITY	NO. OF CONTRACTS	ANNUAL LEVEL	PERCENT OF FUNDS
TOTAL:	49	$9,934,745	100
I. Prevention of Cancer	9	$1,791,374	18
II. Incidence and Diagnosis of Cancer	39	$8,040,808	81
III. Treatment of Cancer	1	$ 102,563	1

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY	CONTRACT	DOLLAR LEVEL
I.	Prevention of Cancer	
	Aichi Cancer Center (Japan) (NO1CP23213)	$ 153,798
	California, University of (NO1CP33327)	60,000
	Fundacion para la Educacion Superior (Colombia) (NO1CP33286)	96,566
	International Agency for Research on Cancer (NO1CP43342)	125,000
	Kuakini Hospital and Home (NO1CP61060)	433,085
	Louisiana State University (NO1CP53521)	338,199
	Minnesota, University of (NO1CP33325)	24,361
	Norwegian Public Health Service (NO1CP43247)	33,604
	Research Corporation of the University of Hawaii (NO1CP53511)	526,761
		$1,791,374
II.	Incidence and Diagnosis of Cancer	
	American College of Surgeons (NO1HV90095)	$ 10,000
	Biotech Research Laboratories (NO1CP61011)	32,375
	Boston University Medical Center (NO1CP00003)	35,478
	Bureau of the Census (YO1CP70501)	80,000
	California Department of Health (NO1CP33353)	1,120,400
	California, University of (San Francisco) (NO1CP43383)	150,000
	Cancer Center of Hawaii (NO1CP71006)	66,849
	Case Western Reserve University (NO1CP43201)	24,470
	Center for Disease Control (NO1CP00082)	21,243
	Center for Disease Control (YO1CP70500)	51,720
	Charity Hospital of Louisiana (NO1CP43262)	344,000
	Connecticut Department of Health (NO1CP61002)	427,383
	Council for Environmental Quality (NO1CP00083)	50,000
	Emory University (NO1CP61027)	321,522
	Fred Hutchinson Cancer Research Center (NO1CP61059)	220,000
	Geomet, Inc. (NO1CP53522)	283,000
	Health Insurance Plan of Greater New York (NO1CP43278)	210,000
	International Agency for Research on Cancer (Lyon, France) (NO1CP61056)	15,000
	Iowa, University of (NO1CP43200)	690,000

TABLE II (CONTINUED)

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY	CONTRACT	DOLLAR LEVEL
	Incidence and Diagnosis of Cancer (continued)	
	Israel Center for Registration of Cancer and Allied Diseases (NO1CP33351)	$ 85,937
	Johns Hopkins University (NO1CP61023)	72,811
	Kaiser Foundation Research Institute (NO1CP61057)	57,000
	Litton Bionetics, Inc. (NO1CP61016)	146,338
	Litton Bionetics, Inc. (NO1CP63975B)	13,000
	Medical College of Wisconsin (NO1HB62964)	12,000
	Meloy Laboratories (NO1CP43216)	264,268
	Michigan Cancer Foundation (NO1CP61028)	887,334
	Minnesota, University of (NO1CP43384)	44,060
	Mount Sinai Medical Center (NO1HB62963)	30,000
	National Center for Health Statistics (NO1CP06009)	391,205
	New Mexico, University of (NO1CP33344)	492,360
	Puerto Rico, Commonwealth of (NO1CP43386)	40,000
	Research Triangle Park (NO1CP00118)	100,000
	State of New Jersey, Department of Health (NO1CP61031)	299,000
	Texas, University of (NO1CP71005)	110,261
	Tulane University (NO1CP61058)	126,000
	U. S. Naval Medical Research Unit #2 (Y01CP60502)	70,817
	Utah, University of (NO1CP43382)	350,000
	Westat, Inc. (NO1CP61034)	270,000
		$8,040,808
III.	Treatment of Cancer	
	Information Management Services, Inc. (NO1CP71010)	$ 102,563
	GRAND TOTAL	$9,934,745

TABLE III

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
OFFICE OF ASSOCIATE DIRECTOR		
California, University of (NO1CP33327)	Prevention	$ 60,000
Harvard University School of Public Health (NO1CP71024)	Incidence & Diagnosis	$ 24,977
International Agency for Research on Cancer (NO1CP43342)	Prevention	$ 125,000
BIOMETRY BRANCH		
Aichi Cancer Center (Japan) (NO1CP23213)	Prevention	$ 153,798
California Department of Health (NO1CP33353)	Incidence & Diagnosis	$1,120,400
California, University of (San Francisco) (NO1CP43383)	Incidence &	$ 150,000
Center for Disease Control (NO1CP00082)	Incidence & Diagnosis	$ 21,243
Charity Hospital of Louisiana (NO1CP43262)	Incidence &	$ 344,000
Connecticut Department of Health (NO1CP61002)	Incidence & Diagnosis	$ 427,383
Emory University (NO1CP61027)	Incidence & Diagnosis	$ 321,522
Fred Hutchinson Cancer Research Center (NO1CP61059)	Incidence & Diagnosis	$ 220,000
Fundacion para la Educacion Superior (Colombia) (NO1CP33286)	Prevention	$ 96,566
Geomet, Inc. (NO1CP53522)	Incidence & Diagnosis	$ 283,000

TABLE III (CONTINUED)

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
Health Insurance Plan of Greater New York (NO1CP43278)	Incidence & Diagnosis	$ 210,000
Information Management Services, Inc. (NO1CP71010)	Treatment	$ 102,563
International Agency for Research on Cancer (Lyon, France) (NO1CP61056)	Incidence & Diagnosis	$ 15,000
Iowa, University of (NO1CP43200)	Incidence & Diagnosis	$ 690,000
Israel Center for Registration of Cancer and Allied Diseases (NO1CP33351)	Incidence & Diagnosis	$ 85,937
Kuakini Hospital and Home (NO1CP61060)	Prevention	$ 433,085
Louisiana State University (NO1CP53521)	Prevention	$ 338,199
Michigan Cancer Foundation (NO1CP61028)	Incidence & Diagnosis	$ 887,334
Minnesota, University of (NO1CP33325)	Prevention	$ 24,361
New Mexico, University of (NO1CP33344)	Incidence & Diagnosis	$ 492,360
Norwegian Public Health Service (NO1CP43247)	Prevention	$ 33,604
Puerto Rico, Commonwealth of (NO1CP43386)	Incidence & Diagnosis	$ 40,000
Research Corporation of the University of Hawaii (NO1CP53511)	Prevention	$ 526,761
Utah, University of (NO1CP43382)	Incidence & Diagnosis	$ 350,000
Yale University (NO1CP33235)	Prevention	$ 570,630

TABLE III (CONTINUED)

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
EPIDEMIOLOGY BRANCH		
American College of Surgeons (NO1HV90095)	Incidence & Diagnosis	$ 10,000
Biotech Research Laboratories (NO1CP61011)	Incidence & Diagnosis	$ 32,375
Boston University Medical Center (NO1CP00003)	Incidence & Diagnosis	$ 35,478
Bureau of the Census (YO1CP70501)	Incidence & Diagnosis	$ 80,000
Cancer Center of Hawaii (NO1CP71006)	Incidence & Diagnosis	$ 66,849
Case Western Reserve University (NO1CP43201)	Incidence & Diagnosis	$ 24,470
Center for Disease Control (YO1CP70500)	Incidence & Diagnosis	$ 51,720
Council for Environmental Quality (NO1CP00083)	Incidence & Diagnosis	$ 50,000
Johns Hopkins University (NO1CP61023)	Incidence & Diagnosis	$ 72,811
Kaiser Foundation Research Institute (NO1CP61057)	Incidence & Diagnosis	$ 57,000
Litton Bionetics, Inc. (NO1CP61016)	Incidence & Diagnosis	$ 146,338
Litton Bionetics, Inc. (NO1CB63975B)	Incidence & Diagnosis	$ 13,000
Medical College of Wisconsin (NO1HB62964)	Incidence & Diagnosis	$ 12,000
Meloy Laboratories (NO1CP43216)	Incidence & Diagnosis	$ 264,268

TABLE III (CONTINUED)

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
Minnesota, University of (NO1CP43384)	Incidence & Diagnosis	$ 44,060
Mount Sinai Medical Center (NO1HB62963)	Incidence & Diagnosis	$ 30,000
National Center for Health Statistics (NO1CP06009)	Incidence & Diagnosis	$ 391,205
Research Triangle Park (NO1CP00118)	Incidence & Diagnosis	$ 100,000
State of New Jersey, Department of Health (NO1CP61031)	Incidence & Diagnosis	$ 299,000
Texas, University of (NO1CP71005)	Incidence & Diagnosis	$ 110,261
Tulane University (NO1CP61058)	Incidence & Diagnosis	$ 126,000
U. S. Naval Medical Research Unit #2 (YO1CP60502)	Incidence & Diagnosis	$ 70,817
Westat, Inc. (NO1CP61034)	Incidence & Diagnosis	$ 270,000

SUMMARY REPORT
BIOMETRY BRANCH
July 1, 1976 - September 30, 1977

The three major functions of the Biometry Branch are (1) to produce baseline data on cancer incidence, mortality and survival for use by investigators and administrators to measure their progress in reducing the cancer burden; (2) to conduct research on the etiology of cancer in man; and (3) to provide statistical consultation and support to other research investigators. These functions encompass a wide range of scientific and managerial skills in bringing people and information together. There have been important changes in the leadership of the Biometry Branch during the past year. When Dr. Sidney Cutler retired in 1975, he was replaced by Dr. Leonard Chiazze who contributed a great deal to the Branch activities as Acting Associate Chief. In July, 1976 Dr. Chiazze returned to his position at Georgetown University, and Mr. William Haenszel retired after serving as Chief of the Biometry Branch for several years. Extensive recruitment efforts have resulted in the selection of Dr. Earl Pollack as the new Branch Chief, and Dr. Pollack plans to assume his new position in July, 1977. The position of Associate Chief will be filled after that time.

The cancer Surveillance, Epidemiology, and End Results (SEER) Program has analyzed data on cancer patients diagnosed during 1973-75, and estimates of cancer incidence for the years have been made using projected population estimates. There has been a slight increase in the incidence rate for all forms of cancer combined since the 1969-71 period. An unexpected increase in the incidence of breast cancer during 1974 was noted. Analysis of a recent set of survival data has been completed and has been published in Cancer Patients Survival, Report No. 5. For the first time, adequate data are available to calculate survival rates for black patients, and they are included in this new report. The data show that there have been some improvements in the relative survival rates for all forms of cancer combined since the 1960's, and the survival rates for Hodgkin's disease and acute lymphocytic leukemia have shown significant improvement since the 1950's. Program efforts have been devoted to quality control and training of personnel responsible for acquiring the basic data and to the development of the best data processing procedures for maintaining and updating information on cancer patients at the regional registry level. Two members of the staff participated in a detailed review of data from the breast cancer screening study conducted by the Health Insurance Plan of Greater New York. The results indicated that although the original histologic diagnosis withstood careful review with excellent agreement, the contribution of mammography to the overall screening benefit could not clearly be delineated and was of questionable value for detecting tumors under one cm. in diameter.

Incidence data from the Third National Cancer Survey covering the period 1969-1971 has been published in NCI Monograph No. 41. This is the most recent publication of detailed incidence data since the Second National Cancer Survey was conducted in 1947. Additional information was obtained regarding treatment of the disease, economic costs, personal habits and occupation from a 10% sample of patients covered by the Survey. Total hospitalization

during the 2-year period following diagnosis averaged 28.2 days per patient with an average hospital payment of $2,529. Lifetime tobacco and alcohol consumption of the patients were quantified and significant correlations were found with a number of cancer sites. Occupations and industries were tested for associations with specific cancer sites while controlling for age, sex, geographic location, education and use of alcohol and cigarettes. Analyses of cancer incidence by socio-economic status, marital status, and urban-rural residence are nearing completion. Out-of-hospital costs to cancer patients are currently being analyzed.

Dr. David Byar and his staff of the Clinical and Diagnostic Trials section have continued their research in statistical methods with particular emphasis on those methods appropriate to the analysis of data from clinical and diagnostic trials. During the past year this research has included a study of methods of comparing multiple treatment groups with unequal censoring patterns, development of a method for comparing different staging systems, a new test for exponentiality, development of survival models incorporating covariate information based on the Weibull distribution, the study of methods for choosing the best of several complex survival models for fitting the observed data, investigation of the effects of stratification in randomized clinical trials, and a study of how thresholds should be determined for diagnostic tests. Work was continued on the problem of determining the optimal treatment for patients based on initial covariate information. The section also provides consultative services in statistical and epidemiological methodology in the design, interpretation and evaluation of clinical trials and other studies requiring this kind of expertise. They are currently involved in studies concerned with cancer of the prostate, bladder, lung, thyroid and breast.

The migrant studies program currently consists of four components: Japanese, Colombian, Norwegian and Polish (sponsored by PL-480 funds in Poland). Observations have established the nature of the changes in site-specific cancer risks in these migrant populations. In all populations efforts are directed toward obtaining comparative epidemiologic and supporting pathological data while taking advantage of the experiments of nature represented by migration. The major efforts have been directed to stomach, large bowel and prostate cancer.

During the past year an extensive review has been made of the epidemiology of large bowel cancer. There are large inter-country variations in large bowel cancer incidence ranging from high in the United States and Canada to intermediate in Western Europe and to low in East Europe, Asia, Africa and South America. Large bowel cancer risks among Japanese in the United States have continued to diverge from those in Japan and have increased to approximate the rates of U.S. whites. Mortality rates for migrants from Poland and Norway to the United States, and from Poland to Austria, have shown rises toward the higher rates of native born in both countries. This pattern is different from stomach cancer where migrants tend to display risks similar to those in their country of origin.

The short interval required to express the changed risks in the migrant population and the inter-population variation indicates the importance of environmental factors. Among environmental factors, dietary patterns of high saturated fat and high meat consumption seem to be of importance. The case-control study of Hawaiian Japanese revealed excess risks for persons who regularly ate Western style meals only, and this was true for both Issei migrants and Nisei offspring. Specific food items consumed more frequently by bowel cancer patients were beef and legumes.

The great virtue of the migrant population approach has been its ability to correlate observations over a wide spectrum of environmental exposures in home and host populations which have clarified the following features of stomach cancer: the critical role of exposures in early life in determining the level of risk; the important distinction between intestinal and diffuse types of the disease for descriptive and analytical epidemiology; documentation of intestinal metaplasia as a precursor lesion; and detection of associations with certain classes of foods that had escaped attention in studies confined to sedentes.

An urgent need for more data relating skin cancer to harmful solar ultraviolet radiation has existed since recent scientific reports have warned about the decomposition of stratospheric ozone by nitrogen oxides and chloroflouromethanes (CFM's). Federal regulatory agencies have already recommended bans on the use of aerosol sprays which use CFM's as propellants. In the near future, there will be critical reviews of all pertinent information on the threat of increased human skin cancer due to excess amounts of ultraviolet radiation reaching the earth's surface as its protective ozone shield is depleted. An interagency task force, the Biological and Climatic Effects Research Program (BACER) has provided $840,000 to NCI for the purpose of conducting epidemiologic skin cancer surveys in as many of the SEER Program locations as possible.

In order to provide initiative and support to independent investigators for research related to our Field Studies and Statistics Program, the Biometry Branch has actively participated in the Cancer Research Emphasis Grants (CREG) Program. Ten CREG announcements have been published so far and more are being planned. Eleven new grants have been funded and five more may be initiated by the end of this fiscal year.

Dr. Gart and his group of statisticians have conducted research in mathematical statistics, probability and applied mathematics with special emphasis on development of new statistical methodology which is applicable to the biomedical sciences. Particular subjects of interest are the analysis of experimental data from the chemical carcinogenesis bioassay program, the methodology of analyzing survival curves, and statistical methods in human genetics, cancer screening models and cancer epidemiology, such as ratios of and matched Poisson models. Consulting assistance in statistical methodology and applied mathematics is provided to NCI investigators and to some extent for NCI contractors. Considerable assistance has been provided to the Carcinogenesis Program, the Viral Oncology Program, the Division of Cancer Biology and Diagnosis, the Division of Cancer Treatment and the Food and Drug Administration.

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81) Williams, R.R., Stegens, N.L. and Goldsmith, J.R.: Cancer site associations with occupation and industry from the TNCS interview. J. Natl. Cancer Inst. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04250-11-B

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Policy Planning and Liaison with Other Organizations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators: James Murray, Contract Program Manager, Biometry Branch
David Levin, Senior Investigator, Biometry Branch
Charles Brown, Mathematical Statistician, Biometry Branch
Charles Land, Mathematical Statistician, Biometry Branch

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A review of all Branch activities for the purpose of planning for future programs and needs has been completed. The Branch receives and responds to a significant number of requests for collaboration and/or consultation which originate throughout NCI, NIH and a variety of federal, state, national and international organizations. The review has assisted in establishing a coordinating mechanism for these requests. A review of the SEER Program has resulted in a revised statement of objectives which takes into consideration changing national needs.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The principal objectives are to provide general planning and direction for the scientific program of the Biometry Branch, coordination and collaboration with other major program components of the National Cancer Institute, and active liaison with other national and international organizations.

Methods Employed:

Standard methods of program planning, management, demography, biometry and epidemiology are applied to coordinate the Branch activities and conduct the research projects.

Major Findings:

Development of the National Cancer Program has reinforced the need for an informational network within the United States, with links to international sources of information on cancer. A network of population-based cancer reporting systems providing information on a total population of more than 20 million people has been organized known as Program of Cancer Surveillance, Epidemiology, and End Results Reporting (SEER). The SEER Program coordinated by the Biometry Branch provides a base for comprehensive cancer data program for the United States, including input from cancer centers, and data both for planning and evaluating cancer control activities. In the development of the SEER Program, particular attention has been given to the collection of descriptive information of clinical interest, e.g., referral patterns, diagnostic procedures in assessing the neoplasm, extent of disease, treatment, and end results. The guidelines being developed for information exchange within the United States should be very helpful in developing guidelines for exchange of compatible data on cancer of an international level. A review of the SEER Program has resulted in redefined objectives of the program in light of current national needs.

Three sections, Demography Section, End Results Section, and Field Liaison Section, are jointly responsible for implementing the SEER Program. The Automated Data Processing Section is intimately involved in helping the participants in the SEER Program organize or reorganize their data processing procedures to facilitate optimum utilization of the collected information.

In order to provide initiative and support to independent investigators for research related to our Field Studies and Statistics Program, the Branch has developed a Cancer Research Emphasis Grant (CREG) Program under the supervision of Dr. James Murray. Ten CREG announcements have been published on the following subjects:

DCCP-4	Epidemiology of Cancer of the Esophagus
DCCP-12	Cancer Epidemiology in Collaboration with the NCI Program of Cancer Surveillance, Epidemiology and End Results (SEER)

DCCP-14 Etiology of Cancer in Special Populations
DCCP-15 Pathology and Epidemiology of Specific Cancer Sites
DCCP-21 Reasons for Variation in Cancer Patient Survival by Race
DCCP-22 Cancer Epidemiology in Collaboration with the NCI Program of Cancer Surveillance, Epidemiology and End Results (SEER)
DCCP-30 Premorbid Psychological Factors as Related to Cancer Incidence
DCCP-31 Studies of Populations at Low Risk for Cancer
DCCP-32 Reasons for Variation in Cancer Patient Survival by Race
DCCP-33 Cancer Epidemiology in Collaboration with the NCI Program of Cancer Surveillance, Epidemiology, and End Results (SEER)

Eleven new grants have been funded so far in this Program and five more may be initiated this fiscal year. Dr. Murray has published a set of median survival times for colorectal cancer patients which take into consideration all important prognostic factors. An analysis is currently underway of the long-term cancer mortality experience as related to tobacco use of approximately 300,000 U. S. veterans in a cohort initially defined in 1953.

Dr. David Levin has worked on the SEER Program and on problems of environmental factors in the etiology of cancer, measuring time trends in cancer mortality, and studies of migrant populations. He has worked with the NCI Information Office on educational material about cancer, and is serving as American coordinator for the US-USSR joint monograph on cancer epidemiology. Dr. Levin is a member of the Interagency Collaborative Group on Environmental Carcinogenesis; he has worked with several other areas within NIH including the Special Virus Cancer Program's Immunology-Epidemiology section and researchers from the Medicine Branch of NCI. He has worked with the staff of the NCHS in analysis of cancer data. He has served on the mathematics faculty of the NIH Federal City College Upward Mobility Program.

During the past year, Dr. Charles Brown has been primarily involved with the problem of the evaluation of human risk from environmental carcinogens. This work has been directed at the chemicals tris, saccharin, and others and he has consulted with Bates and Springer at the FDA, Albert and McGaughy at the EPA, Gaylor at the NCTR, Hoel at the NIEHS, representatives from Canada (Somers, Grice) and the U. K. (Elton), as well as the NCI Carcinogen Bioassay Program (Cueto, Chu). In addition, he is a consultant to the EPA Carcinogen Assessment Group and is a member of the Project Advisory Group for an FDA asbestos bioassay study. He has also been involved in a study of gastric cancer in Colombia, in the evaluation of retinoids as an anti-bladder cancer agent, in consulting on statistical problems with Squire and Levitt of the Experimental Pathology Branch, in a study of the epidemiology of lung cancer, in a study of the survival of patients with colorectal cancer, and in a joint US-USSR project on the rehabilitation of breast cancer patients. Dr. Brown is also involved with aspects of the SEER Program, is a member of the Epidemiology Committee of the Breast Cancer Task Force, and consults with other members of the Biometry Branch on the development of new techniques of statistical methodology.

A large part of Dr. Charles Land's work has been in the area of radiogenic cancer. With Drs. Gilbert Beebe and Hiro Kato of the National Research Council-National Academy of Sciences (NAS-NRC) and the Radiation Effects Research Foundation (RERF), a manuscript is being completed relating cancer and other mortality to radiation dose among Japanese A-bomb survivors. The study was recently discussed with Sir Edward Pochin, who will summarize its findings for the UN Subcommittee on Effects of Atomic Radiation, and with the NAS-NRC Advisory Committee on the Biological Effects of Ionizing Radiation (BEIR). Dr. Land's work on breast cancer incidence and risk among A-bomb survivors has resulted in the completion of two manuscripts, and its relevance to the practice of mammography has resulted in his making a number of formal presentations. Audiences have included the USA-USSR Third Meeting on Cancer Control and Cancer Centers, the Board of Scientific Advisers to the DCCR, the Board of Scientific Advisers to the Bureau of Radiological Health (BRH) of the FDA, the BEIR Committee, the project directors for the Breast Cancer Detection Demonstration Project, the D. C. Medical Society, and the Society for Radiation Research. Informal consultations have been held with a group of Brazilian oncologists, through Dr. O'Conor's office, and Dr. Geoffrey Howe of NCI Canada. Written presentations have been made to the director of the NCI, and to the Upton committee on radiation risk from mammography. The availability of supporting data on women demonstrating increased breast cancer risk as a result of medical exposure has led to a collaborative project with Drs. Roy Shore and John Boice of NYU and BRH, respectively, to calculate improved estimates of radiogenic breast cancer for U. S. women. Lengthy negotiations have been pursued with officials of RERF for a cooperative epidemiological study of the interaction of radiation and other breast cancer risk factors in A-bomb survivors. A study of latent period for radiation-induced leukemia, breast cancer, and lung cancer in A-bomb survivors was presented at the Third International Symposium on Detection and Prevention of Cancer; a second manuscript is being prepared. Dr. Land is NCI project officer for a BRH study of thyroid cancer incidence among patients exposed as children to iodine 131 thyroid uptake tests. He is a member of a committee advising ERDA on studies of cancer mortality among Hanford plutonium workers, and has prepared (with Dr. Blot) an analysis of available data to investigate whether or not certain reported radiation effects could be ascribed to confounding of dose with age or date of death. He also belongs to an FS&S group investigating benefits and risks from mammography, is a consultant to the National Commission on Radiological Protection, and is a member of the BEIR committee.

Dr. Land's work unrelated to radiation includes a collaborative study of dose response for lung cancer mortality and exposure to coal tar pitch volatiles among U. S. coke oven workers, with Dr. Redmond of the University of Pittsburgh. He has also prepared for Dr. Newell an evaluation of epidemiological evidence linking saccharin consumption and risk of bladder cancer.

Significance of the Program to the Institute:

The development of data resources and the provision of statistical consultation services are necessary contributions to the National Cancer Program.

Proposed Course of Project:

Collaboration and consultation with other offices and organizations will continue as in the past. Planning efforts will be directed toward development of an optimum mixture of data resources. Consideration is being given to supplementing the SEER Program with a program of sampling from an enlarged geographic base in order to more effectively provide information needed for planning and evaluating the Cancer Control Program and the International Cancer Research Data Bank Program.

Publications

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Correa, P., Cuello, C., Duque, E., Burbano, L.C., Garcia, F.T., Bolanos, O., Brown, C.C. and Haenszel, W.: Studies of gastric cancer in Colombia III - The natural history of precursor lesions. J. Natl. Cancer Inst. 57: 1027-1036, 1976.

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Squire, R.A., Sporn, M.B., Brown, C.C., Smith, J.M., Wenk, M.L. and Springer, S.: Histopathologic evaluation of the inhibition of rat bladder carcinogenesis by 13-cis-Retinoic acid. Cancer Research. In press.

Land, C.E.:

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Robertson, T.L., Kato, H., Gordon, T., Kagan, A., Rhoads, G.G., Land, C.E., Worth, R.M., Belsky, J.L., Dock, D.S., Miyanishi, M. and Kawamoto, S.: Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: coronary heart disease risk factors in Japan and Hawaii. Am. J. Cardiol. 39: 244-249, 1977.

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Levin, D.L.:

Chiazze, L., Jr., Silverman, D.T. and Levin, D.L.: The cancer mortality scare -- Problems of estimation using monthly data. JAMA 236: 2310-2312, 1976.

Murray, J.L.:

Silverman, D.T., Murray, J.L., Smart, C.R., Brown, C.C. and Myers, M.H.: Estimated median survival times of patients with colorectal cancer based on experience with 9,745 patients. Am. J. Surg. 133: 289-297, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04254-03-B

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cancer Surveillance, Epidemiology, and End Results Reporting (SEER) Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Lillian M. Axtell, Statistician (Health), BB, NCI
Harvey Geller, Head, Field Liaison Section
Max H. Myers, Head, End Results Section, BB, NCI
Theodore Weiss, Head ADP Management Section, BB, NCI
John L. Young, Jr., Head, Demography Section, BB, NCI

Other: See attached sheet

COOPERATING UNITS (if any)

See attached sheet

LAB/BRANCH

Biometry Branch, FS&S Program, DCCP

SECTION

Office of the Chief/ADP Management/Demography/End Results/Field Liaison

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
20	15	5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on cancer patients diagnosed during 1973-75 were submitted to the NCI by nine participants in January, 1977. Estimates of cancer incidence for these years were made using projected population estimates. An unexpected increase in the incidence of breast cancer during 1974 was noted. Cancer patient survival data were submitted by four participants in September, 1975. Analyses of these data showed that there have been some improvements in the relative survival rates for all forms of cancer combined since the 1960's. Program efforts have been devoted to quality control and training of personnel responsible for acquiring the basic data and to the development of up-to-date data processing procedures for maintaining and updating information on cancer patients at the regional registry level.

PHS-6040
(Rev. 10-76)

Other Professional Personnel:

Ardyce Asire, Statistician (Health), BB, NCI
Betty J. Cicero, Medical Record Librarian, BB, NCI
Roger R. Connelly, Research Statistician, BB, NCI
Jack Cunningham, Medical Record Librarian, BB, NCI
Mary M. Cusano, Computer Specialist, BB, NCI
Lynn A. Gloeckler, Statistician (Health), BB, NCI
Benjamin F. Hankey, Mathematical Statistician, BB, NCI
John W. Horm, Statistician, BB, NCI
James A. Larson, Senior Computer Systems Analyst, BB, NCI
William I. Lourie, Jr., Statistician (Health), BB, NCI
James A. Murray, Contract Program Manager, BB, NCI
Constance L. Percy, Statistician, BB, NCI
Philip Prorok, Mathematical Statistician, BB, NCI
Joseph Scotto, Senior Health Services Officer, BB, NCI
Evelyn Shambaugh, Statistician (Health), BB, NCI
James M. Stump, Senior Computer Systems Analyst, BB, NCI
Valerie Van Holten, Computer Specialist, BB, NCI
Paulette White, Mathematical Statistician, BB, NCI

Cooperating Units:

California State Department of Health
University of California, San Francisco
Charity Hospital of Louisiana
Connecticut State Department of Health
Fred Hutchinson Cancer Research Center, Seattle
Research Corporation of the University of Hawaii
University of Iowa
Michigan Cancer Foundation
University of New Mexico
University of Utah
Yale University
Emory University
Geomet, Incorporated
Bureau of Labor Statistics
Commonwealth of Puerto Rico Department of Health

Project Description

Objectives:

To provide descriptive data on cancer incidence and to investigate the variations in risk for a standard range of demographic variables including region, place of residence, race, nativity, economic status, and occupation; to identify promising leads and observational settings for epidemiological investigations to test specific hypotheses on etiological relationships; to evaluate patient survival for such characteristics as extent of disease at therapy, type of therapy, and over time.

Methods Employed:

The descriptive incidence data come from a group of population-based registries covering defined geographic areas which record for each cancer pertinent data on a standard list of characteristics including age, race, sex, place of residence, cancer site, histologic type, and periodically produce statistical reports and supply data on individual patients in accordance with a uniform code to the Biometry Branch. Some participants include epidemiologic research units which develop specific epidemiologic hypotheses suggested by an analysis of morbidity and mortality data. They develop study protocols and may pretest the instruments for data collection. From these preliminary investigations, proposals are generated which can be submitted for financial support either by government agencies through grants or contracts, or by nongovernmental institutions or agencies. The full investigation may be a cooperative activity involving a number of SEER Programs. Active follow-up is either in force or planned for the entire population for end results evaluation.

In order to assure complete, accurate and comparable data among the population-based registries supplying basic data to the SEER Program, an extensive program of training and quality control procedures is employed. Training is carried out in a variety of ways depending on the need and those being trained. Periodic workshops, attended by resprsentatives from all areas, are conducted to introduce new procedures or to reinforce existing data abstracting and coding rules and conventions. Other workshops are conducted at a local level to train new employees or to up-grade the level of abstracting by hospital tumor registrars who contribute to the central registries. Quality control consists of matching of cases identified from a variety of sources, such as pathology lists, radiotherapy rosters, autopsy reports, oncology department patient files, etc., against the registry file to determine completeness of case findings. Accuracy and comparability are evaluated by reabstracting or recoding a sample of the cases, and checking these against the codes on file. Differences are investigated and corrective procedures instituted.

Extensive use of computer edit programs are employed on the data submitted to NCI. This includes a check to insure that all codes are within the bounds specified. Items within the abstract are compared, such as date of birth with age, sex with specific site, site with histologic type, etc. For patients who had more than a single cancer, items between abstract are compared to insure consistency. These edit programs produce printouts presenting the entire

case as well as a message as to the item in question, which are sent back to the program for correction.

Major Findings:

Data for cases diagnosed between January 1, 1973 and December 31, 1975 were submitted to NCI in January, 1977. Two participants did not submit data for 1973. While it is felt that certain areas do not have complete reporting for all years, the data were deemed to be sufficient to calculate incidence rates to permit geographic comparisons by primary cancer sites.

Currently, data are available on 191,437 cases among residents of the nine SEER areas who submitted data. Of these 173,201 cases were malignant lesions, 14,643 were in situ lesions and 3,593 cases were reports of benign tumors which were specifically reportable to the program. Preliminary analyses of these data have centered around the malignant lesions.

Preliminary estimates of the incidence of invasive cancer excluding nonmelanoma skin cancer for all SEER areas combined for 1973, 1974, and 1975 respectively are 313.3, 325.2 and 313.0 per 100,000 population adjusted to the 1970 population of the United States. This compares to a rate of 300.0 per 100,000 for the nine areas reporting to the Third National Cancer Survey 1969-1971. The increase in 1974 was due primarily to an increase in breast cancer reported among white females during that year: 89.8 per 100,000 females compared to 78.2 in 1973 and 82.5 in 1975. Also, in 1975 there was a decline of about 4% in the incidence rates for males. This decline is primarily due to the absence of data from Veterans Administration Hospitals which due to legal restrictions did not report data to the SEER Program.

The rates cited above were calculated based on staff projections of populations of the various SEER areas. Currently, the Census Bureau through an interagency agreement is developing official population estimates by sex and age for whites and nonwhites for each county in the United States. When these estimates become available the preliminary rates are expected to change. These rates will also be expected to change both when data become available from VA Hospitals and as two areas correct the underreporting of their data.

Hence, the 1973-1974-1975 data served as an additional basis for quality control and field audit investigations. By comparing the trend in data over the three year period, as well as among the programs, items for investigation of possible errors or underreporting were identified.

Two program participants and NCI staff have continued collaboration on the development of a sophisticated data management system for collecting and maintaining patient and disease oriented information on a large scale. Information, detailed plans and computer programs will be shared between these groups. System components with documentation will ultimately be available to other SEER participants and to other NCI programs with similar data handling requirements. Progress this year includes the completion of comprehensive system design documentation and computer programs for a core system which will be operational in one SEER Registry late this year.

To assist in the training program and to provide guidance to SEER Program participants and others concerned with cancer registration, a number of publications have been developed and distributed. These include manuals that set out clear guidelines for collecting and coding the data to be submitted to NCI, as well as booklets that can be used for on-the-job training of personnel responsible for carrying out the functions required for data extraction and coding. The demand for these publications have been large and mailings have been made to a variety of medical personnel and facilities.

Four participants in the SEER Program who had formerly been a part of the End Results Program submitted data on cancer patient survival to the NCI in September, 1975. According to these data, there have been some improvements in the relative survival rates for all forms of cancers combined since the 1960's. 1/

	Year of Diagnosis	
	1960-66	1967-73
White		
1-year	61	64
3-year	45	47
5-year	40	41*
Black		
1-year	50	54
3-year	33	37
5-year	28	32*

*Complete 5-year follow-up not available for cases diagnosed 1970 or later.

While these improvements are not large, they are nevertheless improvements. However, there is a potential fallacy in examining survival rates for all forms of cancer combined. If there had been an increase over time in the relative frequency of forms for which survival was poor, a no-increase trend for all forms combined would suggest that there are some types of cancer for which survival has improved. This has in fact happened since there has been an increase in the relative frequency of lung cancer from 1960-66 to 1967-73 from 11 to 13 percent in white patients and 12 to 15 percent in black patients.

For many cancer sites, there have been improvements in the 5-year relative survival rates (i.e., rates adjusted for normal mortality expectation) for both black and white patients. (See attached table.) The greatest improvements among black patients were for those with cancers of the breast, prostate,

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1/The relative survival rate is defined as the ratio of the observed survival rate for the patient group to the rate expected of persons in the general population similar to the patients with respect to age, sex, race and calendar year of observation.

Survival Results From Cancer
Patient Survival, Report No. 5

	5-year Relative Survival Rate (%)			Percent Localized		
	1950-59	1960-66	1967-73	1950-59	1960-66	1967-73
Whites						
Corpus	72	73	77	71	79	79
Breast	61	64	65	43	48	48
Cervix	60	58	58	53	52	44
Bladder	54	58	63	69	79	77
Prostate	47	53	59	52	62	62
Colon	43	44	46	39	42	41
Rectum	40	39	44	44	47	47
Stomach	12	12	12	17	20	18
Lung	7	9	9	17	19	17
Pancreas	1	2	1	14	16	14
Blacks						
Corpus	45	37	47	51	52	51
Breast	43	47	51	30	31	32
Cervix	49	46	53	32	41	39
Bladder	27	28	34	44	51	53
Prostate	38	42	54	43	52	54
Colon	31	35	35	27	32	31
Rectum	23	29	31	30	33	34
Stomach	9	9	14	9	12	17
Lung	5	6	6	12	17	16
Pancreas	1	1	3	13	10	10

Source: Cancer Surveillance, Epidemiology and End Results (SEER) Program, Biometry Branch, National Cancer Institute

bladder, and rectum; and among the white patients mainly for those with cancers of the bladder and prostate. While it may appear that these improvements in overall survival could be attributed to "earlier diagnosis" (increase in percent diagnosed while localized) and, therefore, a statistical artifact, improvement in management must be considered when there has not been an increase in the percent localized. For example, among white men with prostatic cancer the 5-year survival rate increased from 47 to 53 to 59 percent over the three time periods. Correspondingly the percent localized changed from 52 to 62 percent during the first two time periods and then remained at 62 percent for the latest time period.

Survival for Hodgkin's disease has been steadily improving since the 1950's as indicated by the following 5-year relative survival rates for white patients:

1950-69	1960-66	1967-73
34	42	61

A most encouraging trend in survival has been noted for acute lymphocytic leukemia as indicated by the following 3-year relative rates for white patients:

	1950-59	1960-66	1967-73
All ages	2	9	27
Children <15	1	12	34

Significance of the Program to the Institute:

Continuing information on incidence from representative groups of the U.S. population is required so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variations in cancer incidence with respect to geographic and demographic characteristics of the population and changes over time, specific etiologic hypotheses will emerge for testing which may lead to the identification of controllable risk factors. Continued recording of cases will also provide a data base that could be useful in the evaluation of cancer control activities. These data could provide the base-line against which changes resulting from specified cancer control programs could be measured to determine the effectiveness of these programs in reaching their stated goals.

Proposed Course of the Project:

1. During the development phase of the SEER Program activities, intensive effort will be expended to assure that the data being submitted to NCI are of the quality and completeness required for an effective cancer surveillance network. Once this level of reporting is reached, the quality control activity will be geared toward the needs of the individual programs and will depend on each ones level of data quality. The Field Liaison Section staff in cooperation with the Training staff of the University California Medical School in San Francisco will conduct this program of quality control visits, as well as national and regional workshops, intensive formal training programs and will continue to develop various kinds of instructional materials.

2. A study of the cost associated with abstracting and coding the detailed extent of disease and diagnostic procedures data is underway. This cost must be compared with the cost for acquiring summary extent of disease data. Results from this study will provide some guidance regarding implementation of detailed coding schemes for cancer sites other than colon, rectum and breast which are already in force. Coding instructions have been developed and field tested for lung, stomach, uterine cervix, uterine corpus and prostate. Similar descriptive schemes will soon be available for melanoma of the skin, bladder and the lymphomas.

3. The objectives and SEER Program modus operandi have been reviewed by an NCI staff group comprised of consultants drawn from the sections of the Biometry Branch. This group has considered in detail the objectives of the SEER Program vis-a-vis NCI program requirements. Currently an RFP is being developed to solicit a contractor to study alternative methods and/or supplementary sources to SEER's present program to produce data concerning cancer incidence, cancer therapy, extent of disease and associated survival, trends in such data, and national estimates. Further an NCI wide committee consisting of staff responsible for development and management of the various patient-related data systems including SEER has been formed to review the needs of the NCI and the NCP with respect to basic patient data systems and their interface and overlap.

1. Axtell, L.M., Asire, A.J. and Myers, M.H. (Eds.): Cancer Patient Survival, Report No. 5. U.S. Department of Health, Education, and Welfare, Public Health Service Publicaton No. (NIH) 77-992, Washington, D.C., Government Printing Office, 1977. (IN PRESS).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04257-20-B

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Therapeutic End Results and Prognostic Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.H. Myers	Head, End Results Section	BB NCI
	L.M. Axtell	Statistician (Health)	BB NCI
	B.F. Hankey	Mathematical Statistician	BB NCI
	P.C. Prorok	Mathematical Statistician	BB NCI
OTHER:	W.I. Lourie Jr.	Statistician (Health)	BB NCI
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Connecticut Tumor Registry
Charity Hospital of Louisiana
State University of Iowa Hospital
Health Insurance Plan of Greater New York
Israel Center for Registration of Cancer and Allied Diseases

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
End Results Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	6	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Review data from the breast cancer screening study conducted by the Health Insurance Plan of Greater New York indicated that: 1. the original histological diagnosis withstood careful review with excellent agreement; 2. that the contribution of mammography to the overall screening benefit could not clearly be delineated and was of questionable value for detecting tumors under 1 cm. in diameter.

Prognosis for women under 25 who develop breast cancer is strikingly poorer than for women of older ages.

Review of data on patients with main stem bronchus cancers indicated results contrary to previous beliefs: if the tumor was localized to the main stem bronchus, survival for patients with resected cancers was similar to that for patients with resected cancers limited to a single lobe of the lung. Thus surgery should not be ruled out for patients with cancer limited to the main stem bronchus.

Research on statistical methodology for multivariate evaluation of survival data has led to significant improvement in utility of procedures originally developed by Cox. This model requires much more realistic assumptions than previously developed procedures.

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs for the collection and analysis of data on cancer in human populations with special emphasis on the end results of treatment. To cooperate in international studies for the purpose of examining the relationship of etiologic and prognostic factors in cancer. This includes consultation and advice on methodological problems, development of improved statistical tools for the analysis of observations on human populations, and active collaboration in the collection and analysis of data in a continuing collaborative program and in specific projects.

Methods Employed:

1. The End Results Section staff provides advice on the development and application of uniform definitions and procedures to participating cancer registries, provides consultation on planning and implementing special studies, analyzes data submitted to the National Cancer Institute in accordance with a uniform code, and explores the use of more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data.

2. Standard statistical and actuarial techniques are utilized along with accepted principles for records management. Research is also done on the development of new statistical methodologies for analyzing patient survival data and, also, methodologies related to the design of cancer screening programs and the analysis of data collected by these programs.

Major Findings:

1. Work has continued on the development of theory for the design and evaluation of screening programs for the early detection of disease. Effort has focused on the theory of repetitive screening in the context of a randomized controlled trial. Equations are being derived which allow for varying accuracy of the screening examination at different screens. Improvements have been made in estimators of screening parameters. An analysis and comparison of various measures of the effectiveness of a screening program was initiated. With regard to the design and implementation of screening trials, considerable effort was directed toward the Colon Cancer Control Study at the University of Minnesota, which is funded through a contract from DCBD, NCI. An ongoing pathology registry and review process and a death review procedure have been made part of the study. In addition, the trial was designed to provide information on the optimal interval between screens by the inclusion of randomized groups screened at different frequencies.

2. In cooperation with Dr. Noel Weiss of the Fred Hutchinson Cancer Research Center, a study was undertaken to determine whether curative surgery should be attempted in patients with cancer of the main stem bronchus. End Results Group data were used to identify 111 resected cases of cancer arising in the main

stem bronchus and 2465 cases arising in the upper, middle or lower lobe of the lung and diagnosed during 1968-1971. The survival of these two groups was compared using life table methods and relative survival ratios. Survival was comparable both for the overall groups and for subgroups defined by race, sex, age, histologic type and extent of disease. From the available data, it was not possible to assess the role of resection in improving survival; a randomized trial would be necessary for this purpose. However, it was concluded that it is not now advisable to deny resection to patients with operable main stem bronchial tumors (except oat cell tumors).

3. A study of the relationship between laterality and prognosis in ovarian cancer was undertaken in cooperation with Dr. Noel Weiss of the University of Washington. Patients with ovarian cancer identified during 1969-73 were analyzed using life table methods and relative survival ratios. The results indicated that in primary ovarian cancer, neither the stage of disease at diagnosis nor patient survival is related to which of the two ovaries is first involved. Women with cancer in both ovaries have lower survival rates than women with unilateral cancer, though if the disease is still limited to the ovaries at the time of diagnosis, this difference is small.

4. A study was undertaken to estimate median survival times of patients with colorectal cancer in cooperation with Dr. Charles Smart of the University of Utah and other members of the Biometry Branch, NCI. From the End Results Group data file, 9745 colorectal cancer patients diagnosed during 1965-1971 and classified as adenocarcinoma NOS were selected to evaluate the joint effect of various prognostic factors on the median survival time (MST). A multiple regression technique based on the logistic model was used to estimate the MST while taking into account the joint effects of various patient/disease characteristics. A technique was developed which enables a physician to estimate the MST for a patient with a given set of patient/disease characteristics. The results were summarized in tables, allowing the physician to bypass the calculation and refer directly to the MST. These results will aid the physician in determining whether a patient is at sufficiently high risk to warrant adjuvant therapy. The survival experience of this broad historical group of patients with varying prognostic factors can also serve as a basis for comparison of results of studies on highly selected patients treated in various experimental ways.

5. In a letter dated October 31, 1975, Dr. Guy Newell, Acting Director, NCI, established three working groups to review the risk-benefit relationship of including mammography in the screening of asymptomatic women for the early detection of breast cancer. Two of the End Results Section Staff served on one of the three groups, the Pathology Working Group. This group's task was to review gross and microscopic findings of breast cancer found in the HIP screening study, with emphasis on evaluating the role of mammography. Slides were collected for 83% of the cases diagnosed in the first five years of the study and a blind, randomized slide review was conducted. Subsequently, data on tumor size, lymph node involvement, treatment and survival were abstracted from available pathology reports. Analysis of these data led to the following conclusions:

1. There was excellent agreement in histologic interpretation between the HIP coordinating pathologist and the pathologists of the Pathology Working Group.

2. In the reported data from the HIP Study, mammography was negative for 59 (44.7%) of the 132 cancers diagnosed at screening. Therefore, a negative mammogram provided no assurance that a patient did not have breast cancer.

3. In the HIP Study, mammography was an ineffectual means of detecting minimal breast cancer. There were only 7 "minimal" (<1 cm.), infiltrating cancers among those cases reviewed. Five occurred in the Study Group. None was detected by mammography.

4. Since 9 (47%) of 19 infiltrating cancers in the pathology review that were initially detected only by mammography were larger than 2 cm. in diameter, it seems reasonable to expect that many should have been detected by skilled physical examination.

5. In screening women for breast cancer in the HIP Study, it appears that "expert" mammography was performed as contrasted to "routine" physical examination.

6. Very little of the mortality difference at 7 years between the Study Group and Control Group was due to the more frequent diagnosis of in situ (non-infiltrating) cancer in the Study Group. Screening appeared to reduce short-term mortality primarily by detecting infiltrating breast cancer at an earlier stage.

7. Neither the published HIP data nor the pathology review data are completely satisfactory for establishing the independent effect of mammography on survival or mortality rates. The HIP Study was not designed to answer this question.

8. The observations made in this report along with factors not considered, e.g., the cost of mammography and the risk of subsequent radiation-induced breast cancer, raise serious questions about the use of mammography in screening asymptomatic women.

6. In cooperation with the American Joint Committee's task force for staging of soft tissue sarcomas a fascicle has been prepared based on a field trial of 1215 cases. In developing the staging scheme that will be published, a significant correlation of survival with histologic grading was uncovered. Consequently, grading was utilized in developing the staging scheme, a significant departure from the American Joint Committee "tradition." Publication of the supporting scientific paper giving more detailed information is scheduled in the June 1977 issue of CANCER.

7. In cooperation with the Breast Cancer Cooperative Group a study of recurrence after diagnosis of primary breast cancer has been carried out. Three hundred and ninty patients were diagnosed and treated in participating institutions according to the study protocol. The data collected on these patients

included clinical characteristics, pathological characteristics of the primary tumor and lymph nodes as provided by a pathology laboratory which reviewed all slides, and urinary steriod levels. For premenopausal women, obesity, degree of differentiation of the primary tumor, the presence of blood vessel invasion in the primary tumor, nuclear grade of the primary tumor, tumor size and lymph node status were found to be related to recurrence. For postmenopausal women only tumor size, nuclear grade and lymph node status were related to recurrence. The data were further analyzed by an appropriate multivariate technique in order to assess the joint effects of the various factors on recurrence and, using a stepdown procedure, to identify those factors important in predicting recurrence when all factors were considered simultaneously. For premenopausal women, tumor size, degree of differentiation, blood vessel invasion and lymph node status were found to be important whereas for postmenopausal women only tumor size and lymph node status were found to be important. It is suggested that the equations resulting from the multivariate analysis might be useful in identifying premenopausal patients at high and low risk of recurrence even in the absence of lymph node data and could therefore be useful in making adjuvant therapy decisions.

The urinary steriod data has also been analyzed. None of the steroids when analyzed independently has been found to have an important relationship with breast cancer recurrence.

8. Research in statistical methods has led to the further generalization of a multivariate procedure for analyzing survival data proposed by D.R. Cox in 1972, referred to as the proportional hazards regression model. The primary advantage of the model as originally proposed was that it did not require assumptions regarding the nature of the change in the force of mortality over time; however, its use previously has been limited to continuous data, i.e., data in which there are very few ties in the survival times. The model has, therefore, been generalized such that it can be used to analyze grouped survival time data and this will substantially increase its potential for use in analyzing data on large numbers of patients.

The more general model has been used in considering the previously reported black-white difference in breast cancer patient survival. SEER data on 11,442 breast cancer patients, 1,125 being blacks, were analyzed and it was concluded that the survival difference is largely explained by racial differences in extent of disease and demographic characteristics at diagnosis.

9. Data from the SEER program indicate that very young women (<25) with breast cancer have a much poorer prognosis than older women. Furthermore, breast cancer patients 25-34 years of age did not survive as long as women 35 and over, even after adjustment for deaths due to causes other than breast cancer. The patients were all diagnosed between 1960 and 1969, before adjuvant chemotherapy was introduced into general practice.

Significance to Biometric Research and the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the

potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigtions and cancer registries. An example of the interaction between epidemiologic and end results investigations are studies of prognostic factors and the role of these factors in cancer etiology.

Proposed Course

1. This project is part of the collaborative Program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER Program) of the Biometry Branch. Emphasis will continue to be placed on obtaining and reporting data on cancer therapy and associated survival patterns. The SEER Program has currently been expanded to include registries covering the incidence of cancer in defined populations; therefore, studies of the association of end results and the incidence of cancer will be possible for defined populations.

2. Activities in the development of special studies on prognostic factors will continue. In some cases these studies will be in collaboration with the American Joint Committee for Cancer Staging and End Results Reporting, in other cases with cancer specialty centers such as Memorial Hospital (N.Y.C.). With the development of the Resource for Cancer Epidemiology associated with the California Tumor Registry and the Research Unit in Connecticut, the potential for an enlarged special study program will be greatly increased.

3. Activities will also continue on the development of methodology for the design and analysis of screening programs. New models for the analysis and comparison of screening designs and measures of effectiveness will be developed. The role of length-biased sampling in the analysis and interpretation of screening data will be studied in depth. It is anticipated that the HIP breast cancer screening study, a contract of the Biometry Branch, will continue through at least 15 years of follow-up of all breast cancer cases, and that the results of modelling research will be applicable to the HIP data.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04258-09-B

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of Cancer Incidence and Related Etiologic Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators: John L. Young, Jr., Dr.P.H., Head, Demography Section
Haitung King, Ph.D., Senior Research Scientist, Demography Section

Other Investigators: Roger Connelly, Research Statistician, Demography Section
Susan Devesa, Statistician, Demography Section
Dave Levin, Senior Investigator, Biometry Branch
Frances Locke, Statistician, Demography Section
James Murray, Contract Program Management Officer, Biometry Branch
Constance Percy, Statistician, Demography Section
Joseph Scotto, Senior Health Services Officer, Demography Section

COOPERATING UNITS (if any) Aichi Cancer Institute, Nagoya, Japan; Kuakini Hospital, Honolulu; Cancer Registry of Norway; Universidad del Valle, Cali, Colombia; Franingham Field Unit, NHLI; Breast Cancer Demonstration Project, DCCR, NCI; Temple University School of Medicine; World Health Organization, Geneva

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
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NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	4	3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work during the past year has included the following activities: Studies of migrant populations has continued and their contributions to the development of large bowel cancer epidemiology has been summarized; the relationship between cancer and living habits have received attention and is reported under the general heading of cancer risk and life style; a case-control study of reserpine and breast cancer has been reported; a protocol for the classification and staging of pancreatic cancer has been prepared; staff has participated in development of the Ninth Revision of the International Classification of Diseases --Neoplasm Section; staff had responsibility for analyzing data on ultraviolet radiation and skin cancer, a project initiated because of the Department of Transportation's concern over the depletion of stratospheric ozone. Work has begun with the World Health Organization to analyze trends in cancer mortality in forty countries.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisons between high- and low-risk populations; an example of the latter being certain migrant groups in the U.S. useful for the study of host and environmental effects in specific cancer sites; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagnosis, or successful treatment of cancer.

Method Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

Major Findings:

The Migrant Studies Program currently consists of four components: Norwegian, Japanese, Colombian and Polish (sponsored by PL-480 funds in Poland). Observations have established the nature of the changes in site-specific cancer risks in these migrant populations. In all populations efforts are directed toward obtaining comparative epidemiologic and supporting pathological data while exploiting the special capabilities of each collaborator and to take advantage of the experiments of nature represented by migration. The major efforts have been directed to stomach, large bowel and prostate.

Large Bowel. During the past years an extensive review was made of the epidemiology of large bowel cancer, including the contributions made by migrant studies. Major points covered are: a) There are large inter-country variations in large bowel cancer incidence ranging from high in the U.S. and Canada, to intermediate in western Europe, to low in east Europe, Asia, Africa and South America. Presumption of real inter-country differences is strengthened by findings on many migrant groups. Large bowel cancer risks among Japanese in the U.S. have continued to diverge from those in Japan and increased to almost approximate the rates of U.S. whites. Mortality rates for migrants from Poland and Norway to the U.S., and from Poland to Austria, have shown rises toward the higher rates of native born in both countries. This pattern is different from stomach cancer where migrants tend to display risks similar to those of their country of origin.

b) The short interval required to express the changed risks in the migrant population and the interpopulation variation indicates the importance of environmental factors. Among environmental factors, dietary patterns of high saturated fat and high meat consumption seem to be of importance. The case control study of Hawaiian Japanese revealed excess risks for persons who regularly ate Western style meals only, and was true for both migrants and

Nisei offspring. Specific food items consumed more frequently by bowel cancer patients were beef and legumes. Analysis of comparable studies among Japanese in Japan is near completion.

c) Anatomic location of the tumor within the large bowel is of increasing interest as important to study. There is evidence for some differences in incidence rate patterns among countries for the colon, sigmoid and rectum. Among Japanese migrants, there has been a shift in location of tumor from the lower rectum towards upper rectum and rectosigmoid. Also there are differences between colon and rectum in high and intermediate risk countries, and differences by socioeconomic class.

d) Autopsy studies have shown strong evidence that intestinal polyps are a precursor of large bowel cancer. Adenomatous polyps have increased among migrant groups. A promising area for future case control studies is to focus on the epidemiology of intestinal polyps and other suspect antecedent conditions. Also almost completed is an analysis of pathologic reports on the large bowel cancer cases in the case control studies of Hawaiian Japanese and in Japan.

The great virtue of the migrant population approach has been its ability to correlate observations over a wider spectrum of environmental exposures in home and host populations, which have clarified the following features of stomach cancer: The critical role of exposures in early life in determining the level of risk; the important distinction between intestinal and diffuse types of the disease for descriptive and analytical epidemiology, this does not imply that the final word has been said on the meaning and significance of the type distinctions; documentation of intestinal metaplasia as a candidate precursor lesion; and detection of associations with certain classes of foods that had escaped attention in studies confined to sedentes.

Occupation and Health Among the U.S. Chinese. In the absence of adequate information, no firm conclusion may be drawn of the occupation health profile for the U.S. Chinese. Among the noticeable findings is an extremely high TB rate for the Chinatown in San Francisco where a high proportion of this minority group are restaurant workers. The rates are from three (cases) to four (deaths) times that for the whites in San Francisco. This is in keeping with the elevated mortality rate reported for U.S. males in eating and drinking places. The relatively high rate for cancers of buccal cavity and pharynx shown for the latter group remains to be established among the Chinese counterparts, however.

Cancer Risk and Life Style. The relationship between cancer and living habits was examined and published in accordance with a proposed conceptual scheme shown below:

Ecologic influence
  Geo-Hydro-Atmo-sphere
  Bio-Phyto-Chemo-sphere

Occupational effects
  Job experience
  Socioeconomic stratum

Sociocultural intervention
Macro-societal configuration
Religio-ethnic orientation

There seems to be a strong association between cancer risk and an individual's social status. An association with low socioeconomic status was shown to be quite marked for stomach, lung, scrotum, bladder, skin and cervix. The upper social classes, including U.S. Lutheran, Episcopalian and Presbyterian clergymen studied previously by this Branch, seemed to be vulnerable to cancer of the prostate. These clergymen, along with their U.S. and British counterparts and other learned professionals in both countries also showed an elevated risk for cancer of the intestines.

In matters of marital, sexual and reproductive behavior, a much higher cervical incidence was shown for the married than for single white females, in contrast to an absence of risk differential between married and single Black women. An association was also suspected between high risk of cervical cancer among wives and elevated rates of venereal diseases among their husbands, particularly in those occupations involving extended travel on the road.

The much speculated association between colonic-rectal cancer and beef intake was generally supported by the incidence data of the Third National Cancer Survey, judged by the percent of households using selected meats and fish in various parts of the country.

The usefulness of life style concept for the study of cancer risk was also demonstrated by the risk differential among various religio-ethnic groups (Protestant, Catholic and Jewish; Adventists; Mormons; the Amish; U.S. Chinese and Japanese) in relation to their distinguished living habits.

The Case Control Study of Reserpine and Breast Cancer is a joint project of NCI, NHLI and ACS. A report in Lancet (September 21, 1974) suggested an association between mammary cancer and this drug commonly used to treat hypertension. The purpose of this study is to gather further data to test for this association as requested by the directors of NCI, NHLI and FDA.

From 18 of the 27 Breast Cancer Demonstration Project Centers, 543 newly diagnosed breast cancer patients were matched by age, race, location and screening date with 478 biopsy negative controls and 1,428 completely normal controls (total 2,449 ladies). Questionnaires have been mailed to all subjects to request information on the diagnosis and treatment of hypertension or edema, as well as the name and address of prescribing physicians who are also polled. All questionnaires have been coded and computerized along with data items from the original screening center entry visit. A thorough analysis of the reserpine issue is nearing completion and the results will be available shortly.

Classification and Staging of Pancreatic Cancer. The Pancreatic Cancer Task Force of the American Joint Committee for Cancer Staging and End Results Reporting has been working for several years on the problem of classifying and staging cancers of the pancreas. Under NCI staff supervision a protocol for gathering data on patients with this disease has been developed. A

pretested protocol with abstracting instructions was finalized in late 1975 and data have now been gathered on about 600 cases. The coding of these data has been completed on 200 of these cases and detailed analyses will begin as soon as the additional 400 are coded and computerized.

International Classification of Diseases for Oncology (ICD-O). After NCI field tested and edited ICD-O, the book was published by the World Health Organization. ICD-O is a coded nomenclature designed for coding neoplasms by topographic site, histologic type, behavior and grading. The importance of ICD-O is that it provides for the first time an internationally acceptable and agreed upon system for coding neoplasms. This book is a supplement to ICD-9, which has also just been published by WHO and NCI scientists have helped revise the neoplasm section of this volume, as well as the ground rules pertaining to cancer published herein, which should greatly increase the comparability of cancer statistics.

World Health Organization. Collaboration has begun with WHO to analyze trends in cancer mortality from 1950-1974. Data are availalbe from approximately 30 countries for the entire time period and an additional 10 for 15-year periods. Missing are data for U.S.S.R., the People's Republic of China and most of Africa. Computer tapes containing number of deaths and populations by sex and age for fifteen cancer sites have been sent from Geneva and are currently being analyzed.

Staff have also been working with the WHO in the development of an Expert Committee on Cancer Information Systems through working groups which have met or will be meeting in Minsk, U.S.S.R. in September, 1976 and Geneva in June, 1977.

Increase in U.S. Cancer Deaths. There has been an increase in the absolute number of cancer deaths in the United States. This observed increase over time in the number of cancer deaths in the United States could be attributed to not only a real increase in population risk due to changing environmental factors but also to the growth in size of population as well as a shift in its age distribution. The contributions of each of these three factors have been quantified. The results indicate that the latter two factors have had a much greater role than the first in producing the 180% increase in number of deaths due to cancer during the forty year period 1930 to 1970, and that the real increase in age-adjusted cancer mortality rates has been 0.5%, or 0.24% per year, part of which is certainly due to better diagnosis and reporting.

Nonmelanoma Skin Cancer Epidemiologic Survey. An urgent need for more data relating skin cancer to harmful solar ultraviolet (UV-B) has existed since recent scientific reports have warned about the decomposition of stratospheric ozone by nitrogen oxides and chloroflouromethanes (CFM's). Federal regulatory agencies have already recommended bans on the use of aerosol spray cans which use CFM's as propellants. In the near future, there will be critical reviews of all pertinent information by government as well as individual interests concerned with the threat of increased human skin cancer due to excess amounts of UV-B reaching the earth's surface, as its protective ozone shield is depleted.

An interfederal agency task force, the BACER (Biological and Climatic Effects Research) program, provided $840,000 as pass through funds from its lead agency, the EPA (Environmental Protection Agnecy), to the NCI for the purpose of conducting epidemiologic skin cancer surveys in as many of NCI's SEER Program locations as possible. Under this cooperative interagency agreement between the EPA and the NCI, the NCI for its part will use EPA funds to partially support the supervision and conduct of a special morbidity survey as follows: 1) Nonmelanoma incidence studies at several SEER locations to supplement four of the earlier TNCS sites. 2) Pilot study on nonmelanoma patients in TNCS study in Minneapolis to determine exposure habits, skin types, etc. 3) If pilot study indicates that it is appropriate then conduct case/control studies to determine ethnic background, skin pigmentation, and exposure habits of skin cancer patients and controls.

Seven SEER locations have expressed their willingness to participate in this study (Seattle, San Francisco-Oakland, Utah, New Mexico, New Orleans, Atlanta and Detroit). We expect the study to begin in these locations by June 1, 1977.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, ascertains trends in risks which may reflect the action of various etiologic agents, identifies high- and low-risk population groups which can be studied and compared, and provides observations on human populations useful for correlation with laboratory studies. Ad hoc investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

The migrant population studies represent a major biometric effort of the Institute. If these studies elucidate the environmental factors involved in the incidence of certain forms of cancer and indicate those which are predominantly environmental and those which are predominantly genetic, it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project:

The migrant studies will emphasize analysis of data bearing on the pathogenesis of the major sites under study. The findings from the case-control studies of gastrointestinal cancers and diet will be used in developing the protocols for the ongoing cohort study of Hawaii Japa ese males and the extension to cohorts in Japan. Field studies using the gastrocamera and other screening techniques of the population at very high risk for stomach cancer in one department of Colombia are receiving high proprity. Greater efforts in the synthesis of data in hand and being collected are required and this will require closer collaboration with the principal investigators for the several related programs being supported by research contracts. The skin cancer survey, reserpine study and world cancer mortality trends analysis should progress as outlined above.

The initiation of new projects is not predictable since many are undertaken as the need becomes apparent and as staff time is available. A number of studies are in various phases of completion, and the items listed below represent long-term efforts and shortage of staff resources will delay completion until at least the following year.

Critical review of epidemiologic research in China will be made periodically to provide suggestive clues for potential collaborative studies and guidelines for possible field trips. A comparative study of cancer risk among the Chinese will be initiated. Plans for a study of cancer and living habits among the Chinese in this country and other areas are being made.

New studies of clerical mortality patterns will be initiated in order to establish more firm evidence on cancer risk in this profession. The final results would be useful for initiation of similar studies on cancer in other learned professions which has been greatly neglected.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04260-17 B

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Consultation on Clinical Trials

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. P. Byar	Head, Clin. & Diag. Trials Section	BB NCI
OTHER:	M. H. Gail	Medical Statistical Researcher	BB NCI
	S. B. Green	Medical Researcher	BB NCI
	J. A. Koziol	Staff Fellow	BB NCI
	L. R. Muenz	Mathematical Statistician	BB NCI
	D. K. Corle	Computer Systems Analyst	BB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
Clinical & Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to provide consultative services in statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials and other studies requiring this kind of expertise. For some trials the section provides full statistical support including development of detailed study plans, randomization of patients, collection, processing, editing, and analysis of data, and preparation of scientific papers. At present members of the section are involved in studies concerned with prostate cancer, bladder cancer, lung cancer, thyroid cancer, and breast cancer. Other major consultative activities include membership on advisory committees, grant and contract funding review committees, and review of scientific articles concerned with clinical trials.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To provide consultative services in statistical and epidemiological methodology in the design, interpretation and evaluation of clinical trials. A major responsibility during the past year has been a continued role in analyzing data generated by the clinical trials of prostate and bladder cancer conducted by the Veterans Administration Cooperative Urological Research Group (VACURG). During the past year the section has undertaken to provide full statistical support for two new trials. The first is concerned with immunotherapy in stage I lung cancer, and the second with treatment of rapidly growing inflammatory breast cancer in Tunisia. Other major projects have included the continued analysis of data on prostate and bladder cancer, new analyses of large sets of data on lung and thyroid cancer, and continuing progress in developing a central data bank for patients with breast cancer studied in projects funded by the Breast Cancer Task Force Treatment Committee.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New techniques are developed by the personnel working in the Section to handle specific problems (see Project No. Z01-CP-04409-02-B).

The most important projects of the Section will be described separately. Generally in consultations on clinical trials members of the Section assist the investigators in developing detailed study protocols, in determining the numbers of patients necessary for the study, in deciding what data should be recorded and at what intervals in time, assist in developing forms for the recording of data and advise on proper methods of analysis of the final data or undertake these analyses themselves. Such assistance is generally acknowledged in the publication of findings by the medical investigators.

VACURG Studies of the Genital-Urinary Cancer

As explained in last year's progress report, the VACURG has been involved in six clinical trials of treatment for genitourinary cancer. In May of 1976 grant support for these projects was terminated. However, members of the Section are heavily involved in the analysis of this very large set of data. Two studies concerned with bladder cancer were relatively small, but combining all studies we have information on over 4,500 patients with prostate cancer.

Prostate Cancer

During the last year in collaboration with Drs. Mostofi and Harada of the Armed Forces Institute of Pathology we have undertaken a major study of histological variables in prostatic cancer. Some 1,000 cases were reviewed

in detail and the importance of histological features was assessed against the survival of the patients. In the past year two papers were published concerning the prognostic significance of biochemical measurements performed at the time of diagnosis and the response of these measurements to treatment. The first of these papers was concerned with plasminogen and fibrinogen and a second with cholesterol and triglycerides. During the past year another paper concerned with serum haptoglobin has been submitted for publication. Work has continued on assessing radiological evidence of distant metastases and the correlation of these findings with survival. In the past year we have learned that although histology is an excellent prognostic factor in the early stages of disease, it is virtually uncorrelated with the prognosis in the late stages. Extent of metastasis as measured by an ordinary bone survey, however is quite useful for late prognosis. This information should be helpful in designing clinical trials for advanced disease. A new paper is currently in preparation comparing patients who were randomized to radical prostatectomy vs. placebo for stage I and II disease. Although few patients have died from cancer, measures of progressive disease such as increased acid phosphatase, appearance of bone metastases, or increased size of the primary lesion are being assessed for this comparison. As far as we are aware, this is the only randomized comparison of surgery vs. no therapy in early prostatic cancer and thus should provide important information unavailable elsewhere.

Although the VACURG lost its funding near the end of the last fiscal year, intensive efforts have been devoted to preparing a new protocol and grant proposal for the study of chemotherapy in previously untreated patients in high stage-grade categories. If this project is funded the Clinical and Diagnostic Trials Section will serve as the statistical office for this study. Another project related to prostate cancer during the past year has been an historical comparison of the VACURG experience to the series of patients treated by radiotherapy at three other centers (Stanford University, Washington University at St. Louis, and the Peter Brigham Hospital in Boston). Preliminary comparisons have been made to the series from Stanford adjusting for tumor stage, tumor grade, and the age of the patient. These studies should be useful in sharpening questions to be asked in future prospective randomized trials of radiotherapy vs. hormone treatment.

Bladder Cancer

During the past year it has been possible to extend the results from the first bladder study, that comparing radiotherapy vs. surgery vs. their combination for stage II and III patients, to include ten year survival figures. These have been submitted for publication. In addition we have continued to analyze data from the second bladder study comparing placebo, pyridoxine (vitamin B6), and intra-vesicle instillation of thiotepa in patients with stage I bladder cancer. The results of this study indicated that thiotepa was probably more useful than placebo, but the most interesting finding was that a subset of the patients may definitely be helped by pyridoxine. These results have been so interesting that they have stimulated a group in England to begin a new trial comparing placebo and pyridoxine with

the inclusion of measurements of tryptophane metabolism in order to try to identify the subset of patients who may benefit from vitamin therapy.

Data Center for Breast Cancer Studies

Last year at the request of the Breast Cancer Task Force, the Section undertook to establish a data center for studies of breast cancer. Initially this program was requested by the Treatment Committee of the Task Force for three purposes: (1) to compare surgical adjuvant studies for early breast cancer; (2) to relate clinical data to estrogen receptor measurements performed in their core laboratory in Worcester, Massachusetts; and (3) to collect clinical data to be correlated with biochemical marker data. Considerable work was accomplished on this project during the past year and at present we have data on some 550 women with breast cancer who have also had estrogen receptor studies performed. It is anticipated that this number will grow considerably in the next year. Also, plans are being completed for collecting data on large number of women for evaluation of biological markers for breast cancer in cooperation with the Marker Group of the Breast Cancer Task Force, a group comprised of members from both the Treatment and Diagnosis committees. Centers for participation in this project were selected in May 1977 and data acquisitions should begin in the coming year. Serum samples for all women will be stored in a central reference laboratory for use in evaluating new markers. The Section will be responsible for the collection, editing, and analysis of all data, and for providing a continually updated inventory of material in the serum bank. It is anticipated that this project will be continued for a number of years and that members of the Section will be continually involved with the collection and interpretation of these data.

Analysis of EORTC Data

As explained in last year's progress report, Dr. Byar assisted the program of the European Organization of Research on the Treatment of Cancer (EORTC) by means of a series of consultations to their Data Center in Brussels. In order to help their Data Center catch up on some of their backlog, data from a study of treatment of advanced bronchogenic carcinoma involving some 250 patients has been analyzed in the Section. These data were reported at an EORTC meeting last year in Florence and a report is currently being prepared for publication. In addition, this Section has undertaken the analysis of data on some 1,400 patients with thyroid cancer, obtained from an EORTC registry begun in 1968. The goal in studying these patients is to identify prognostic factors and then to use them to make adjusted treatment comparisons. It is anticipated that the results of these adjusted comparisons will be useful in suggesting hypotheses to be resolved in future randomized clinical trials.

Surgery vs. Radiotherapy in Early Node-Positive Breast Cancer

There is continuing controversy regarding the use of radiotherapy in early breast cancer. Most clinicians seem to agree that it is useful in preventing

local recurrence but there is sharp disagreement regarding the effect on survival. A variety of randomized trials have shown a slight tendency for earlier distant metastases among patients receiving radiotherapy, but levels of statistical significance have not been impressive.

For some two years Dr. Muenz has been the statistician of a cooperative international trial comparing surgery with and without radiotherapy. Surveillance of the patients' immunological status has been added in the hope that it will permit definition of classes of patients who are likely to benefit from radiotherapy and those for whom radiotherapy is not advisable.

Intake into the trial has now ended with the admission of 300 patients, more than half of whom have been followed for at least two years. Dr. Muenz will analyze the data to see if lymphocyte counts, erythrocyte sedimentation rates, and immunoglobulin levels differ among those who have and have not suffered distant dissemination.

Inflammatory Carcinoma of the Breast in Tunisia

The acronym for the inflammatory carcinoma seen in Tunisia is PEV (poussee evolutive) meaning "agressively evolving". The diagnosis of PEV has always been based on strictly clinical criteria and the disease has yet to be put on a solid histopathological basis. Before leaving Lyon Dr. Muenz prepared a study of the comparative survival of patients with breast cancer but distinguishing those with and without PEV. The goal was to show that patients with comparable levels of disease severity nonetheless have different prognoses depending upon whether or not they have PEV.

After examining the survival experience of 412 patients followed between two and seven years, Dr. Muenz concluded that delay in diagnosis and treatment did not explain the sharply different prognosis for patients with PEV compared to other breast cancer patients with a similar TNM classification. Results of this study are to be published in Cancer.

Dr. Muenz is the statistician for a breast cancer trial now beginning in Tunisia. His association with this project grew out of work he did while stationed in Lyon, France during 1975 and 1976. The trial compares two modes of therapy for a form of breast cancer (PEV; see above) similar to inflammatory carcinoma. However, inflammatory carcinoma represents about 3% of cases seen in the U.S. but nearly 60% of cases seen in Tunisia. The form detected there is characterized by edema and rapid tumor growth as well as inflammation.

The trial compares a three-drug combination (CMF) and surgery for local tumor control with the same three-drug combination but using radiotherapy for local control. All patients receive multiple courses of CMF, are then randomized to either surgery or radiotherapy, and then receive several more courses of drug therapy. Admission criteria include presence of the Tunisian form of inflammatory carcinoma and absence of any evidence of metastatic disease. The protocol has been completed, study forms have

been designed, and randomization slips prepared awaiting entry of the first patient. One-hundred patients will be randomized and followed until death or three years after randomization.

Trials for Lung Cancer

Dr. Gail is statistician for the Lung Cancer Study Group which includes the Mayo Clinic, M.D. Anderson Tumor Hospital, University of California School of Medicine (UCLA), Virginia Mason Clinic, Vanderbilt University, and Princess Margaret Hospital. This group has the potential to recruit over 200 stage I lung cancer patients per year. A double-blind trial of intrathoracic BCG immunotherapy against conventional therapy in stage I patients with resected disease is nearly ready to begin admitting patients. Two other protocols are being refined. One is a three arm comparison of radiotherapy, radiotherapy plus levamisole, and conventional therapy in stage II and III resectable patients with squamous cell tumors. The other is a study comparing cis-platinum, adriamycin, BCG, and levamisole in stage II and III resectable patients with adenocarcinoma or large cell carcinoma.

Sickle-Cell Crisis Trial

Dr. Gail is acting as statistician for a controlled clinical trial of carbamide (urea) prophylaxis against sickle cell crisis. Approximately 50 homozygous S patients have been recruited in Ghana for this study which began in September, 1976. The therapy has been well-tolerated, and the data have been promptly reported, but no statistically significant differences in crisis rate have been demonstrated to date. Follow-up is expected to last at least another 18 months.

Hyperthermic PAM for Stage III-A Melanoma

In 1974 Dr. Muenz was a coauthor with Dr. John Stehlin, and others, on a paper regarding the use of phenylalanine mustard (PAM) as a heated perfusate in melanoma therapy. Survival of 67 patients was compared with that of historical controls obtained from M.D. Anderson Hospital. Those receiving the heated perfusate lived much longer than those receiving the same treatment but unheated. Now three more years of follow-up information has been obtained and Dr. Muenz has been asked to reanalyze the data.

An International Standard for the Radioimmunoassay of Alpha-Fetoprotein

Alpha-fetoprotein (AFP) is obtained from the amniotic fluid of women whose fetuses have certain congenital defects or from the serum of people with various liver diseases including primary hepatic cancer. It has been used for some time as a diagnostic and prognostic tool. In order to establish uniform criteria for medical intervention (e.g., abortion) it is necessary that there be agreement on the level of AFP titer at which intervention is desirable. This, in turn, requires that AFP titers be comparable from one laboratory to another and this can only be done by use of an international standard preparation.

Such an international preparation could be used directly for parallel line bioassays or it could be used to calibrate local standards which then could be used to do the same sort of assays. Dr. Muenz has concluded an international collaborative study on the use of a proposed AFP standard preparation. The work has resulted in a paper to be published in the Journal of Biological Standardization and two invitations to address international conferences.

The basic conclusion is that AFP measurements using radioimmunoassay are only reliable and reproducible if the titer is sufficiently high. Several medical conditions, which might potentially require intervention, nonetheless give rise to AFP titers so low as to make diagnosis very risky.

Other Consultative Activities

Dr. Green has been collaborating with Dr. Marvin Schneiderman on an analysis of the relationship between 1-year and 5-year survival rates for various cancer sites. The goal is to elucidate what relationship exists, and to indicate what predictive value there is in the 1-year data.

During the preceding two years Dr. Green was involved in the design, implementation, and analysis of a population-based study of referral, diagnosis and treatment patterns for childhood acute lymphocytic leukemia. During the past year, additional follow-up analysis was performed. Also, the design of a similar study for female breast cancer was begun.

Anorexia and wasting in cancer patients hastens death and diminishes the efficacy of systemic treatments. Dr. Muenz has consulted on a set of trials considering the use of TPN (Total Parenteral Nutrition) in both adult and pediatric patients. The goal is to find those cancers and those treatments which would be most usefully influenced by intensive feeding.

The National Cancer Institute is sponsoring a multi-center study regarding the psychological state of women after mastectomy designed to determine if psychotherapeutic intervention might help women better adjust to the consequences of mastectomy. Psychological profiles will be determined on 1,500 women, 500 within one year of mastectomy, 500 within one year of heart surgery or acute heart disease, and 500 with no acute or chronic disease. By examining the "normals", baseline levels for attitudes regarding self and family will be determined. These will be compared with attitudes of women in the two disease states. Dr. Muenz is assisting in the design of this study.

The Environmental Protection Administration (EPA) is proposing a new set of regulations to determine the acceptable levels of E. Coli contamination in drinking water. E. Coli is used as an indicator to signal the presence of pathogenic organisms. The EPA regulations, while intuitively reasonable, lack a theoretical justification.

Dr. Muenz has studied the sampling characteristics of the EPA rules and developed a theoretical model of E. Coli distribution, finding that the EPA rule has a low probability of signalling contamination even when the density of E. Coli is relatively high. Dr. Muenz delivered a paper on this topic at the EPA and proposed other possible sampling procedures with superior characteristics.

Dr. Gail served as special consultant to the Committee on Science and Technology (U. S. House of Representatives Ninety-Fourth Congress) in its evaluation of the Community Health and Environmental Surveillance System of the Environmental Protection Agency. A congressional report on this subject appeared in November, 1976.

Dr. Byar is a member of the National Prostatic Working Cadre and of the Treatment Committee of the Breast Cancer Task Force. Both of these projects require reviewing extramural projects for National Cancer Institute funding.

Dr. Gail serves as a consultant to the Immunodiagnosis Committee of the NCI. He supervises statistical evaluation of promising immunodiagnostic tests for this committee, and he advises the Committee in its deliberations.

Dr. Green serves as consultant to the Committee on Cancer Immunotherapy, chaired by Dr. William Terry. This committee oversees many contracts dealing with various aspects of immunotherapy, involving both animal studies and clinical trials, relating to a wide variety of cancer types. Dr. Green acts as advisor on all aspects of experimental design and analysis and is involved in all stages of the Committee's work (initial awarding of contracts, review of on-going studies,and site visits).

Significance to the Program of the Institute

The variability of the course of cancer in individual patients means that the assessment of treatment differences, determination of the usefulness of diagnostic tests, or the proper interpretation of data from observational studies are often statistical problems. Members of the Section are frequently consulted for advice or collaboration on such problems. Besides the projects listed above, there are numerous other short-term consultations dealing with specific studies, proposal reviews, site visits, and review of manuscripts submitted for publication. Some of the consultations (e.g., Breast Cancer Task Force, Immunodiagnosis Committee, Immunotherapy Committee) involve extensive trials which represent considerable efforts of the Cancer Program. The Section represents an important resource for expert assistance in study design, implementation, and statistical and computer analysis of studies being carried out by many other groups, including those within NCI (e.g., DCCP, DCCR, DCT), within other parts of the Federal Government (e.g., EPA, Congress), and international cancer agencies (IARC, EORTC).

In any healthy research environment, individuals skilled in many disciplines are necessary. The Section provides expertise in statistical matters relating

to the study of cancer in humans. The ability to provide meaningful consultation is greatly enhanced by having three M.D.'s in the Section who are also well-equipped statisticians. Members of the Section are also skilled in computer applications to analysis of medical data.

A further advantage to the Institute is that actual day-to-day experience as a statistical support center for several large-scale clinical trials and active involvement in consultation on other projects provides an ideal environment for identifying important methodological questions of general applicability to the design, conduct, and analysis of clinical trials.

Proposed Course of the Project

In the coming year we plan to continue our involvement in the studies described above. In addition much planning and effort will be required to increase the scope and utility of the Breast Cancer Data Bank. We shall continue to maintain a staff of biostatisticians capable of meeting the needs of the Institute for statistical advice and collaboration.

Publications

Byar, D. P. and the Veterans Administration Cooperative Urological Research Group: VACURG Studies on Prostatic Cancer and its Treatment. To appear as Chapter 13 of the Atlas of Genitourinary Pathology, Vol. 1, ed. Myron Tannenbaum (in press).

Day, N. E., Muenz, L. R. and Tulinius, H.: Some aspects of familial breast cancer. To appear in the Proceedings of the Conference on Breast Cancer sponsored jointly by the Deutsche Krebsforschung Gesellshaft and the National Cancer Institute, Düsseldorf, July 1976.

Gail, M. H.: Special Consultant to the Committee on Science and Technology, U.S. House of Representatives, 94th Congress, report entitled: "The Environmental Protection Agency's Research Program with Primary Emphasis on the Community Health and Environmental Surveillance System", 1976.

Green, S. B., Myers, M. H., and Fink, D. J.: A population-based study of referral, diagnostic and treatment patterns for childhood acute lymphocytic leukemia. Am. J. Epidemiology 106: July, 1977 (in press).

Harada, M., Mostofi, F. K., Corle, D. K., Byar, D. P., Trump, B. F.: Preliminary Studies of Histologic Prognosis in Cancer of the Prostate. Cancer Treatment Reports 61: 223-225, 1977.

Jordan, W. P., Blackard, C. E., Byar, D. P., and the Veterans Administration Cooperative Urological Research Group: Reconsideration of Orchiectomy in the Treatment of Advanced Prostatic Carcinoma. Southern Medical Journal (in press).

Muenz, L. R., Sizaret, P. et al.: Analysis of a second collaborative study of radioimmunoassay of alphafoetoprotein. Journal of Biological Standardization (in press).

Seal, U. S., Doe, R. P., Byar, D. P., Corle, D. K., and the Veterans Administration Cooperative Urological Research Group: Response of plasma fibrinogen and plasminogen to hormone treatment and the relation of pretreatment values to mortality in patients with prostatic cancer. Cancer 38: 1108-1117, 1976.

Seal, U. S., Doe, R. P., Byar, D. P., Corle, D. K., and the Veterans Administration Cooperative Urological Research Group: Response of serum cholesterol and triglycerides to hormone treatment and the relation of pretreatment values to mortality in patients with prostatic cancer. Cancer 38: 1095-1107, 1976.

Tabbane, F., Muenz, L. R., et al.: Prognostic and clinical aspects of a rapidly growing breast cancer found in Tunisia. Cancer (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04265-12-B

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Consulting in Statistics and Applied Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Gart	Head, Mathematical Statistics & Applied Mathematics Section, Mathematical Statistician	BB	NCI
	R.J. Connor	Mathematical Statistician	BB	NCI
	M.W.J. Layard	Mathematical Statistician	BB	NCI
	H.M. Pettigrew	Mathematician	BB	NCI
	D.G. Thomas	Mathematical Statistician	BB	NCI
	R.E. Tarone	Mathematical Statistician	BB	NCI
OTHER:	J. Nam	Mathematical Statistician	BB	NCI
	A.M. Smith	Statistician (Health)	BB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to collaborate with NCI researchers on mathematical problems related to many areas of cancer research. Consulting assistance in statistical methodology and applied mathematics is provided for NCI investigators and to some extent for NCI contractors. In general the study is devoted to accelerating the use of quantitative methodology in various aspects of the NCI intramural and extramural program.

PHS-6040
(Rev. 10-76)

Project Description

To collaborate with NCI scientists on the applications of mathematical statistics and applied mathematics to the design, analysis, and interpretation of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research, (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators, and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program and extramural program.

Methods Employed:

The methodology of applied mathematics, mathematical statistics and probability is applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem.

Major Findings:

During this year the staff advised and collaborated with many investigators in the major divisions of research in the National Cancer Institute as well as some outside contractors and other investigators. The various projects are grouped below in terms of the Divisions and Areas of the projects.

Division of Cancer Cause and Prevention

Dr. Gart was invited to discuss the statistical aspects of the evaluation of animal feeding experiments for possible carcinogens before the Data Evaluation Panel of the Cancer Clearing House.

Dr. Layard continued to provide consulting assistance to Dr. Gio B. Gori, Director of NCI's Smoking and Health Program, and to the prime contractor, Enviro Control Inc. He co-authored a chapter "Supplement to the Statistical Analyses of the Third Cigarette Study" for the NCI monograph Toward Less Hazardous Cigarettes. Report No. 3. The Third Set of Experimental Cigarettes.

For Dr. Gio B. Gori, Deputy Director, DCCP, Dr. Layard reviewed the analysis of a carcinogenesis experiment involving the feeding of carrageenan to rats and hamsters.

Division of Cancer Cause and Prevention: Field Studies and Statistics

Dr. Gart and Mrs. Alroy Smith, are continuing the implementation of the theoretical methodology of Dr. Gart on ratios and cross-product ratios for use on the data of Mr. Joseph Scotto of the Demography Section of the Biometry Branch which compares cancer rates among immigrant and native born Americans in various cities.

Mr. Nam advised Dr. Guy Newell, Jr., Acting Director, National Cancer Institute, on the analysis of some incidence data by month of diagnosis. The data were provided by the Third National Cancer Survey (TNCS) and SEER Program. The seasonal pattern of incidence of all lymphosarcomas under 40 years old is the most striking finding in this analysis.

Dr. Connor and Drs. Roger Williams and Manning Feinleib, Epidemiology Branch, NHLBI, have completed their analysis of a case-control study on reserpine usage and breast cancer. A paper reporting on this study is in preparation.

Dr. Gart, together with Mr. Scotto of the Demography Section, advised Dr. Erik Bjelke of the University of Minnesota on the statistical analyses of NCI supported epidemiologic studies being done in Minnesota and Norway.

Mr. Nam is collaborating with Mr. Joseph Scotto of the Demography Section, on the statistical analysis of data of TNCS regarding the possible cyclical phenomenon in incidence for various cancer sites.

Dr. Connor is a member of the Field Studies and Statistics Ad Hoc Breast Cancer Screening Review Group. This was formed to provide NCI with a biostatistical, epidemiologic, mathematical resource for reviewing problems associated with screening for breast cancer and the Breast Cancer Detection Demonstration Program.

Mr. Nam analyzed skin melanoma data of Third National Cancer Survey in collaboration with Mr. Joseph Scotto of the Demography Section. Solar ultraviolet in the UV-B portion of the spectrum is suspected to be the principal carcinogenic agent for skin melanoma. This study investigated the monthly pattern of occurrence of skin melanoma among whites and further subdivides the distribution by age, sex, anatomical site, and geographical area, in order to discern whether such patterns in incidence are in agreement with U.V. patterns. A paper on this research is in preparation.

Dr. Robert Hoover, Environmental Epidemiology Branch, and Dr. Robert Connor have agreed to assume the scientific direction of a case-control study of breast cancer. This study is being done by the Division of Cancer Control and Rehabilitation in conjunction with the Breast Cancer Detection Demonstration Program.

Mrs. Smith provided valuable computing assistance in many of the consultations listed in this project.

Mr. Thomas further improved the section's library of computer programs which are often used in the consulting activities of other members of the section.

Dr. Gart was appointed and served on the Field Studies and Statistics Program Review Group.

Dr. Gart served as a member of the SEER Program Review and Analysis Committee.

Division of Cancer Cause and Prevention: Carcinogenesis

Dr. Gart and Mr. Nam have analyzed the data of two feeding studies of Chlordane and Heptachlor in both sexes of rats and mice. The experiment was conducted by Gulf South and the final pathology report was done by the Experimental Pathology Laboratory under contract to the National Cancer Institute. The result of their statistical analysis is published in two technical reports.

In support of the Chlordane and Heptachlor reports, Dr. Gart testified before an Administrative Hearing of the EPA on the statistical aspects.

Dr. Tarone is collaborating with Dr. Kenneth Chu of the Carcinogen Bioassay and Program Resources Branch regarding the use of historical control tumor experience for purposes of quality control and assessment of laboratory management, and for possible use in the analysis of future carcinogenesis experiments. He was an invited lecturer on these topics at the Applied Statistics Seminar of Carnegie-Mellon University in February 1977.

Dr. Gart is collaborating with Dr. Kenneth Chu and others in the Carcinogenesis Program on a paper describing the statistical analysis of the NCI bioassay survival and tumor incidence data system.

Dr. Pettigrew continued to assist in the development of an interactive computer system to retrieve and analyze data stored in the Carcinogenesis Bioassay Data System, working with Mary Linhart of the Carcinogen Bioassay and Program Resources Branch and Cathy Creede of the Division of Computer Research and Technology.

Dr. Connor is working with Drs. Herman Autrup and Curtis Harris, Experimental Pathology Branch, on research on the metabolic activation of polynuclear aromatic hydrocarbons. In a current study they are comparing the metabolism of benzo[a]pyrene and 7,12-dimethylbenz[a]-anthracene in human pancreatic duct and bronchus explants.

Dr. Tarone continued to assist Dr. Harry Gelboin, Chief of the Chemistry Branch, with the statistical analysis of a twin study designed to determine the degree of genetic determination of Aryl Hydrocarbon Hydroxylase induction levels in cultured human leukocytes. He assisted in preparing a paper based on this study for publication.

Dr. Tarone assisted Dr. Kenneth Kraemer of the Chemistry Branch with the statistical analysis of experiments to quantify the decrease in thymidine incorporation in circulating leucocytes of psoriasis patients treated with Psoralen and ultraviolet light. He also helped to prepare a manuscript detailing the results of these experiments.

Dr. Tarone completed collaborative work with Dr. Friedrich Wiebel of the Chemistry Branch concerning the reproducibility of Aryl Hydrocarbon Hydroxylase induction levels in cultured human lymphocytes. He assisted scientists at the University of Wisconsin with the analysis and interpretation of a double blind study on the measurement of AHH induction levels.

Dr. Connor continues to provide statistical support for research on the effects of chronic immunosuppression on carcinogens that is being done at the University of Utah on a contract from the Biology and Immunology Segment. Data from this project is now being analyzed. It is expected that papers reporting on this work will be completed in the coming year.

Dr. Pettigrew advised Dr. Sherman F. Stinson of the Lung Cancer Branch concerning the presentation of results of a study comparing various automated methods of cell counting with counts made visually by a technician.

Dr. Gart advised Dr. Lionel Poirier of the Carcinogen Metabolism and Toxicology Branch on the statistical analysis of host-mediated assays for possible carcinogens.

Mr. Nam and Dr. Gart continued their statistical analysis of a feeding study in rats of seventeen chemicals based on additional pathology information. They are collaborating on this study with Dr. Elizabeth K. Weisburger of the Carcinogen Metabolism and Toxicology Branch. The experiment was done at Litton Bionetics under contract to NCI.

Dr. Gart is collaborating with Dr. Joseph A. DiPaolo and Mr. Paul J. Donovan of the Biology Branch on the analysis of cell transformation in hamster embryo cells. The mathematical model of one-hit curve is fitted to doses of Benzo[a]Pyrene with and without irradiation. The variation among plates is also examined. A joint paper on the subject is in preparation.

Dr. Connor advised Dr. Berton Zbar, Biology Branch, on the analysis and interpretation of data from animal immunotherapy studies.

Dr. Pettigrew, in conjunction with Dr. Carl Smith of the Carcinogenesis area has continued to provide statistical consultation on the design and analysis of experiments conducted under contract by Dr. Albert Segaloff of the Alton Ochsner Medical Foundation, New Orleans, Louisiana. A manuscript reporting the results of an investigation of synergism between radiation and estrogen administration in mammary tumor induction in the AxC rat is being completed. Further experiments are in progress using this system to investigate the phenomena associated with mammary carcinogenesis, in particular, the apparent protective effect of early pregnancy.

Division of Cancer Cause and Prevention: Viral Oncology

Dr. Layard analyzed data from experiments performed by Dr. U. Heine, Viral Studies Section, to determine the relative increase in lengths of virus molecules under different spreading methods.

Dr. Pettigrew is continuing to advise and collaborate with Dr. Michael Chirigos of the Virus and Disease Modification Section of the Laboratory of RNA Tumor Viruses on a variety of topics.

Division of Cancer Biology and Diagnosis

Dr. Connor is working with Dr. James McCoy, Laboratory of Immunodiagnosis, on the analysis of migration inhibition assays. A paper reporting on a study of Ewing's Sarcoma extracts in migration assays is in preparation.

Dr. Pettigrew has continued his collaboration with the Laboratory of Pathophysiology, providing statistical advice to Dr. Pietro Gullino on a continuing basis.

Dr. Tarone is performing the statistical analysis of a series of radioautography experiments for Dr. Bruce Wetzel of the Dermatology Branch. The purpose of the experiments is to determine the relationship between the phases of the cell cycle and certain morphological characteristics of the cells as determined by scanning electron microscopy.

Dr. Gart has collaborated with Ms. Susan O. Sharrow of the Immunology Branch and Dr. Martin L. Slater of the NIAMD on research in the cell cycle analysis in the growth of Saccharomyces cerevisiae. Mathematical models for the growth cycle were constructed and used to discriminate among various hypotheses. A joint paper has been submitted for publication.

Dr. Layard has analyzed data in a continuing series of experiments performed by Dr. Mearl F. Stanton, Laboratory of Pathology, dealing with the relationship between the dimensions of fibrous materials and induction of mesotheliomas in rat lungs.

Dr. Ronald Herberman, Laboratory of Immunodiagnosis, and Dr. Connor are continuing to collaborate on studies related to immunodiagnosis of cancer. Presently they are concerned with the immunologic monitoring of cancer patients. Also Drs. Herberman and Connor are working with investigators at Litton Bionetics who are doing work under contract for NCI.

Dr. Tarone assisted Dr. Jay Robbins, Mr. Robert Petinga, and Dr. Alan Andrews of the Dermatology Branch with the statistical analysis of experiments designed to determine if typical Xeroderma Pigmentosum complementation group A fibroblasts have detectable ultraviolet light-induced unscheduled DNA synthesis, as measured by radioautographic methods. A paper based on these experiments has been submitted for publication.

Dr. William Pomerance, Diagnosis Branch, and Dr. Connor have continued their collaborative association. Their work together is concerned with projects studying the effectiveness of methods used for cancer screening and diagnosis. Dr. Connor has assisted in the evaluation of current studies and in the development of future ones.

Dr. Pettigrew, in conjunction with Darlene Meyer of the Automatic Data Processing Management Section, continued to advise Dr. Robert Blaese on the storage retrieval and analysis of data on diagnostic tests made on patients of the Metabolism Branch. The data includes such tests as lymphocyte transformation, mixed lymphocyte reactions, and cytotoxicity as well as complete patient history.

Dr. Layard assisted Dr. Alvaro Morales, Laboratory of Immunodiagnosis, with the analysis of data on the reduction in the number of bladder tumors in patients receiving BCG therapy.

Dr. Tarone designed and performed the statistical analysis of experiments for Dr. Katherine Sanford and Ms. Sandra Handleman concerning the cytology of spontaneous neoplastic transformation of cells grown in culture. A paper based on this study has been submitted for publication.

Dr. Tarone continued to aid Dr. Katherine Sanford and Mr. Gary Jones of the Laboratory of Biochemistry with the statistical analysis and interpretation of experiments designed to determine if randomness of locomotion can be used to characterize cell lines which have undergone spontaneous neoplastic transformation. A paper detailing the results of this study has been submitted for publication.

Dr. Tarone analyzed for Dr. Katherine Sanford of the Laboratory of Biochemistry and Dr. Ram Parshad of Howard University a series of experiments designed to study the frequency of occurrence of various forms of chromosome damage in cells exposed to light under different culture conditions.

Dr. Tarone provided statistical analysis to Dr. Ray Gannt and Dr. Katherine Sanford of the Laboratory of Biochemistry in experiments designed to quantify the effect of light upon X ray-induced DNA damage, as measured by the method of alkaline elution. He is assisting with the design of further experiments to verify preliminary findings.

Dr. Connor assisted Linda Engel, Laboratory of Pathology, and Dr. Edward Leonard, Laboratory of Immunobiology, in the analysis of their experimental results.

Dr. Connor is providing statistical support for a lung cancer immunotherapy trial. Drs. Elliot Perlin, National Naval Medical Center, and James Weese, Laboratory of Immunodiagnosis, are the primary investigators for the study. Early results of the trial were presented at the Fourth Annual Chicago Symposium on Immunotherapy of Solid Tumors.

For Drs. Guy Bonnard and Alvaro Morales, Laboratory of Immunodiagnosis, Dr. Layard performed statistical tests for the effectiveness of stimulation of lymphocytes by breast cancer cells in a cytotoxicity study.

Dr. Tarone assisted Dr. Cecil Fox of the Laboratory of Biochemistry with the statistical analysis of experiments designed to determine whether cell surface and nuclear morphological changes are associated with neoplastic transformation. A paper reporting the results of these experiments has been accepted for publication.

Dr. Tarone is collaborating with Dr. Henson of the Laboratory of Pathology on an epidemiological study of the various histological types of cancer in the female reproductive organs. A paper based on this study has been submitted for publication.

Dr. Tarone provided statistical analysis for Dr. William Taylor of the Laboratory of Biochemistry in an experiment to determine the effect of per cent oxygen in phase and initial inoculum size on the plating efficiency of cells. In addition, he analyzed changes in the ratio of lactic acid produced to glucose used as cell lines become neoplastic.

Division of Cancer Treatment

Dr. Layard is providing statistical assistance to Dr. Daniel Von Hoff, Drug Evaluation Branch, in the conduct of a large-scale study of risk factors related to the cardiotoxicity of the anticancer drug Adriamycin. Data on several thousand patients are being collected from several major oncology groups. It is expected that the results of this study will form the basis of the design of prospective studies of the effects of this drug.

For Dr. Matthews Bradley, Laboratory of Molecular Pharmacology, Dr. Layard has performed analyses of a number of experiments to determine *in vitro* survival curves of cell colonies subjected to several anticancer drugs.

Dr. Layard advised Dr. Gary Strauss, Drug Evaluation Branch, on problems of statistical methodology.

For Dr. Daniel Von Hoff, Drug Evaluation Branch, Dr. Layard is examining data on the variation in toxicities of the anticancer drugs in children and adults.

Dr. Layard has performed an analysis of the time relationship between last dose of Daunomycin and onset of congestive heart failure for Dr. Daniel Von Hoff, Drug Evaluation Branch. The results of this study will appear in a letter to the editor of Cancer Treatment Reports.

Miscellaneous

Dr. Gart served as a consultant to a Working Group of the Bureau of Foods of the FDA. He advised the group on the statistical methodology to be used in the evaluation of a animal study of Red Dye No. 40.

Mr. Nam and Dr. Pettigrew carried out a comprehensive review of the evidence from four FDA studies and one Russian study concerning the possible carcinogenic effects of Red Dye No. 2. A report summarizing the results was prepared for Dr. Marvin A. Schneiderman, Associate Director for Field Studies and Statistics, for use in preparing his testimony on Red Dye No. 2. In addition, Dr. Gart, Dr. Pettigrew and Mr. Nam participated with Dr. Schneiderman in several discussions with Mr. Campbell and Mrs. Poplin of the General Counsel's Office, FDA, concerning our results and rebutting the testimony of the statisticians for the Certified Color Manufacturer's Association.

Dr. Connor has advised Dr. Mary Coleman of the Children's Brain Research Clinic in Washington, D. C. on the statistical aspects of a study on neonatal screening for mental retardation.

Mr. Nam reanalyzed the data of Purdon's saccharin study for Dr. Robert S. Gordon, Special Assistant to the Director of NIH. It was found that this sweetener reduces the levels of glucose as well as alpha-2 globulin.

Drs. Layard and Pettigrew reviewed and prepared a report on a proposed mortality study of employees of the Homestake Mining Co. This was submitted to Dr. Jack Butler, Acting Director, Office of Extramural Coordination and Special Projects, National Institute of Occupational Safety and Health.

Dr. Pettigrew advised Dr. William L. Marsh, Department of Pathology, University of Virginia Medical Center, on the proper method of estimating a standard error based on duplicate measurements.

Dr. Connor advised Dr. Reuben Siraganian, Laboratory of Microbiology and Immunology, NIDR on the statistical analysis of his experimental studies.

Dr. Connor assisted in the preparation of a report on the Workshop on Polyamines as Biochemical Markers in the Diagnosis and Treatment of Cancer. This meeting was sponsored by the American Cancer Society.

Dr. Gart advised the staff of the Consumer Product Safety Commission on the statistical interpretation of an NCI animal study of the flame retardant Tris.

Dr. Gart and Mr. Thomas have advised Dr. Charles Anello, Director of the Division of Drugs of the Bureau of Drugs of the FDA on a method for deciding on sample size.

Mr. Thomas has advised several researchers regarding implementation of computer programs. Among them are: Dr. Richard Greenstreet and Mr. Mario Guarracinio, of the Cleveland Clinic; Mr. Lawrence Lusold and Ms. Kitt Hogye, FDA; Mr. Charles W. Riggs, Frederick Cancer Research Center; and Dr. John Diem, Tulane Medical Center.

Reviewing and Refereeing

Dr. Gart served as a referee for Blood and the American Journal of Epidemiology. Dr. Connor served as referee for the American Journal of Public Health. Dr. Tarone served as a referee for the Journal of Toxicology and Environmental Health. Drs. Pettigrew, Layard, Tarone, Connor, and Gart served as referees for J.N.C.I.

Significance to the Problem to the Institute:

Members of this section are assuming an essential role in much research within the National Cancer Institute. Their activities include not only statistical analysis but also planning of valid experiments.

Proposed Course of the Projects:

Several of the projects mentioned in the Major Findings section will continue. In particular the collaboration with the various projects in epidemiology within Field Studies and Statistics, as well as in the Carcinogenesis area, the Division of Cancer Biology and Diagnosis, and other areas will be progressing.

Publications

Bast, R. C., Okuda, T., Plotkin, E., Tarone, R., Rapp, H. J., and Gelboin, H. V.: Development of an assay for Aryl Hydrocarbon [Benzo(a)pyrene] Hydroxylase in human peripheral blood monocytes. Cancer Res. 36: 1967-1974, 1976.

Fox, C. H., Caspersson, T., Kudynowski, J., Sanford, K. K., and Tarone, R. E.: Morphometric analysis of neoplastic transformation in rodent fibroblast cell lines. Cancer Res. 37: 892-897, 1977.

Gart, J. J.: Statistical analyses of the first mouse skin painting study. In Gori, G. B. (ed.): Report No. 1 Toward Less Hazardous Cigarettes. The First Set of Experimental Cigarettes. DHEW Pub. No. (NIH) 76-905, 1976, pp. 110-148.

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Herberman, R. B., Oldham, R. K., and Connor, R. J.: Statistical analysis and selection of baseline controls in cytotoxicity assays. In Bloom, B. R. and David, J. R. (eds.): In vitro methods in cell-mediated and tumor immunity. Academic Press, New York, 1976, pp. 481-488.

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McCoy, J. L., Jerome, L. F., Anderson, C., Cannon, G. B., Alford, T. C., Connor, R. J., Oldham, R. K., and Herberman, R. B.: Leukocyte migration inhibition by soluble extracts of MCF-7 tissue culture cell line derived from breast carcinoma. J. Natl. Cancer Inst. 57: 1045-1050, 1976.

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Perlin, E., Weese, J. L., Heim, W., Reid, J., Oldham, R. K., Mills, M., Miller, C., Blom, H., Green, D., Bellinger, S., Law, I., Connor, R., Cannon, G. B., and Herberman, R. B.: Immunotherapy of carcinoma of the lung with BCG and allogeneic tumor cells. In Crispen, R. G. (ed.): Proceedings of the Fourth Annual Chicago Symposium on Immunotherapy of Solid Tumors. 1977, in press.

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Von Hoff, D. D., Rosencweig, M., Layard, M. W. J., Slavik, M., and Muggia, F. M.: Daunomycin-induced cardiotoxicity in children and adults. A review of 110 cases. Amer. J. Med. 62: 200-208, 1977.

Wetzel, B., Sanford, K. K., Fox, C. H., Jones, G. M., Westbrook, E. W., and Tarone, R. E.: Topography of nonneoplastic and neoplastic cells of common origin. Cancer Res. 37: 831-842, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04267-12-B

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Research in Mathematical Statistics and Applied Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Gart	Head, Mathematical Statistics & Applied Mathematics Section Mathematical Statistician	BB	NCI
	R.J. Connor	Mathematical Statistician	BB	NCI
	M.W.J. Layard	Mathematical Statistician	BB	NCI
	H.M. Pettigrew	Mathematician	BB	NCI
	D.G. Thomas	Mathematical Statistician	BB	NCI
	R.E. Tarone	Mathematical Statistician	BB	NCI
OTHER:	J. Nam	Mathematical Statistician	BB	NCI
	A.M. Smith	Statistician (Health)	BB	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to conduct research in mathematical statistics, probability and applied mathematics and especially to develop new statistical methodology which is particularly applicable to the biomedical sciences. Particular subjects of interest are the methodology of analyzing survival curves, statistical methods in human genetics, cancer screening models, and in cancer epidemiology, such as ratios of and matched Poisson models.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To conduct research in mathematical statistics, probability, and applied mathematical statistics; to develop new statistical methodology which is especially appropriate to bio-medical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability, and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and to test approximations by simulation techniques.

Major Findings:

The Research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability, and applied mathematics. These are summarized below.

1. Statistical Analysis of Dose Response with Life Table Adjustments

Donald G. Thomas and John J. Gart in collaboration with Norman Breslow of the University of Washington have completed work on a comprehensive program for "Trend and Homogeneity Analyses of Proportions and Life Table Data". This work has been invaluable in the analysis of dose response relationships and survival data for carcinogenesis experiments. The program has already achieved widespread acceptance and has been distributed to the FDA, EPA and numerous research and industrial laboratories. It has also been used in the analysis of data involved in hearings at both the EPA and FDA. A paper describing the methodology has been accepted for publication.

2. Error Rates for Carcinogenicity Screens

Robert E. Tarone, in collaboration with Dr. Thomas R. Fears and Dr. Kenneth C. Chu, examined the error rates associated with the statistical analysis of animal carcinogenesis experiments. In response to criticism from pharmaceutical statisticians, they prepared a report which quantified the relationship between the spontaneous tumor rate and the probability of a false positive at a given tissue, and they computed upper bounds on the probabilities of overall false positive results in all tissues. A paper based on these findings has been accepted for publication. Dr. Tarone presented these results in an invited paper at the Eastern Regional Meeting of the Biometric Society (ENAR) at Chapel Hill, North Carolina, in April 1977. John J. Gart served as an invited discussant at this session.

3. Statistical Analysis of Cancer Screening Studies

Robert J. Connor is continuing his investigation of design and analysis problems in trials of cancer screening programs. Currently Dr. Philip Prorok, End Results Section, Biometry Branch and Dr. Connor are preparing a paper on the statistical evaluation of such studies.

4. Analysis of Ratios and Cross-Product Ratios of Poisson Variates

John J. Gart has completed work on a paper which derives tests for ratios and cross-product ratios of Poisson variates, both in simple and stratified (say by age and/or sex) cases. The methods may be applied to the ratios of incidences of rare diseases. The method has been applied to skin cancer and immigrant data. The paper has been invited to appear in a special issue of Communications in Statistics A entitled "Theory and Practice for Categorical Data Analysis".

Alroy M. Smith has written a computer program implementing these methods.

5. Exact Test for Matched Counts

Maxwell W. Layard has completed a study of tests for comparing matched Poisson-distributed counts in crossover designs. These tests have potential applications in the analysis of clinical trials. A paper dealing with this work has been submitted for publication.

6. Models for Mammary Tumor Systems in Rodents

Hugh M. Pettigrew has continued his efforts to develop models for mammary tumor systems in rodents, and is considering extensions of existing statistical theory that may be necessary to evaluate data from such systems, which generate multivariate data subject to censoring.

7. Identification of Cyclical Trend

Jun-mo Nam has expanded his study of the proper statistical methods in analysis of seasonality. The various estimates are compared in the statistical efficiency and several tests are assessed in the power performance.

8. Distribution-Free Tests for Equality of Survival Curves

Robert E. Tarone continued his study of distribution-free tests for equality of censored survival (incidence) distributions. He showed that two statistics commonly used in performing such distribution-free tests differ only in the choice of weights which are functions of the number of individuals at risk at the time of each event. A paper related to this work has been accepted for publication.

9. Approximate Tests for Trends in Bioassay Data

John J. Gart is investigating approximate tests for non-zero slopes, equality of non-zero slopes, and intercepts in the analysis of proportion data in one or several strata. The methods will prove useful in analyzing certain carcinogenesis experiments particularly when the numbers may be too large to use exact methods.

10. Pseudo-Random Numbers from Continuous Distributions

Donald G. Thomas is continuing to improve his program for generating pseudo-random numbers from the Pearson family of frequency distributions. The speed of generation has been increased and the method is now being compared with an alternative method.

This program should be extremely useful in simulation and robustness studies.

11. Mathematical Theory of Epidemics

Hugh M. Pettigrew is continuing his research in the mathematical theory of epidemics with two or more types of infectives. One potential area of application of such models is to family data on leprosy.

12. Statistical Analyses of Carcinogenesis Experiments

Maxwell W. Layard is investigating methods of analysis of animal carcinogenesis experiments when tumors cannot be observed until the death of the animal. In particular, analysis of serial sacrifice designs, and the effects on estimates of dependence between tumor occurrence and death are being considered.

13. Alkaline Elution of DNA

Robert E. Tarone is attempting to obtain a mathematical model for the rate of elution of DNA on polyvinyl chloride filters. The method of alkaline elution provides a sensitive measure of DNA single-strand length distribution, however the physical process appears to be a two-stage procedure, only one of which depends upon strand length. A mathematical model would be useful in the derivation of tests to determine shifts in the distribution of strand length associated with DNA damage.

14. Tests for Dose-Response

Robert E. Tarone continues to study tests for monotone response in experiments in which a chemical is administered at increasing dose levels. In particular, he is examining the relationship between the magnitude of the response under the null hypothesis (the spontaneous response rate) and the power of the tests for trend.

15. Size of Experiment and Detection of Linear Dose Response

Jun-mo Nam has been investigating the sample size needed for detecting a linear dose response in carcinogenesis bioassay study.

16. Statistical Analyses of HL-A Gene Frequency Data

John J. Gart has continued his research into the statistical methodology used in the analysis of ABO-like systems such as the HL-A system. In October he presented a seminar on the subject at S.U.N.Y. at Buffalo.

17. Estimating Inbreeding Coefficient in Codominant Systems

Jun-mo Nam continued his work to investigate the relative efficiency of several estimators of the inbreeding coefficient in codominant genetic systems.

18. Dose Response, Adjusting for Multiple Comparisons

Dr. Gart, Mr. Thomas and Dr. Todd Thorslund of the EPA are working on methods of evaluating in the conditional reference set the true type one error for several schemes of multiple testing of several proportions.

19. Comparison of Several Treatments with One Control in Proportion

Jun-mo Nam has devised asymptotic test for one-several comparison in proportion and investigated some practical limitations of Bonferroni's upper bound. Preliminary computation shows the asymptotic test with correction is satisfactory even for sample sizes of each of three groups as low as ten. He presented some results of this study at The Second Integrated Symposium on Science and Technology, Seoul, Korea, July, 1976.

20. Confidence Limits for Ratios and Differences in Proportions

Donald G. Thomas and John J. Gart published a table of P values and confidence limits for the odds ratio, ratio, and difference in proportions for a wide variety of 2x2 tables. This table will prove useful in sample size determination.

21. Small Sample Properties of a Test for Combining 2x2 Tables

John J. Gart, together with S. H. Li and R. Simon of the DCT have written a paper on the small sample properties of this test. The paper corrects some erroneous conjectures published by other authors.

Reviewing and Refereeing

John J. Gart served as an Associate Editor for Biometrics. Maxwell W. Layard reviewed a grant proposal for the National Research Council. Robert E. Tarone and Maxwell W. Layard served as referees for The Journal of the American Statistical Association. John J. Gart and Robert E. Tarone served as referees for Biometrics. Hugh M. Pettigrew and Robert E. Tarone served as referees for The American Statistician. Maxwell W. Layard served as referee for Mathematical Reviews and Psychometrika.

Significance to the Program of the Institute:

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI, particularly in DCCP, or other workers in cancer research. Others are mathematical models which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects described in the major findings will be continuing, e.g., breast cancer screening models in human, mammary tumor models in rodents, research on censored survival tests, estimation of frequencies and inbreeding coefficients in genetic models, and generation of pseudo-random numbers. Other research, not referred to above, shall also be initiated; it will include the development of new statistical methods and mathematical models in various biomedical problems that come to our attention during the year.

Publications

Tarone, R. E. and Ware, J.: On distribution-free tests for equality of survival distributions. Biometrika 64: 156-160, 1977.

Thomas, D. G. and Gart, J. J.: A Table of Exact Confidence Limits for Differences and Ratios of Two Proportions and Their Odds Ratios. J. Amer. Statist. Assoc. 72: 73-76, 1977.

Thomas, D. G., Breslow, N. and Gart, J. J.: Trend and Homogeneity Analyses of Proportions and Life Table Data. Comp. Biomed. Res. 10: in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04268-09-B

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Third National Cancer Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

John L. Young, Jr., Dr.P.H., Head, Demography Section, Biometry Branch, NCI
Roger R. Connelly, Research Statistician, Demography Section, Biometry Branch, NCI
Susan S. Devesa, Statistician, Demography Section, Biometry Branch, NCI
John W. Horm, Statistician, Demography Section, Biometry Branch, NCI
Constance Percy, Statistician, Demography Section, Biometry Branch, NCI
Joseph Scotto, Senior Health Services Officer, Demography Section, Biometry Branch, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
Demography Section

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	3

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan statistical areas and two states during the period 1969-1971 has been developed and analyzed to determine the incidence of cancer classified according to demographic characteristics of the patient as well as characteristics of the tumor. For a 10% sample of patients in the register, additional information was obtained regarding treatment of the disease, economic costs, personal habits, and occupation. Total hospitalizations per patient during a two-year follow-up period averaged 28.2 days with an average hospital payment of $2,529. Lifetime tobacco and alcohol consumption of the patients were quantified and significant correlations were found with a number of sites. Occupations and industries were tested for associations with specific cancer sites while controlling for age, sex, geographic location, education, and use of alcohol and cigarettes. Analyses of cancer incidence by socioeconomic status, marital status and urban-rural residence are nearing completion. Out of hospital costs to cancer patients are being analyzed.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To prepare a complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-71, plus all other cancer cases seen by doctors or hospitals in those areas; to obtain demographic data and a description of the tumor for each patient; and to study certain economic, social and other aspects of the disease in a sample of patients.

Methods Employed:

Detailed descriptions of the survey methodology are available in previous reports. In brief NCI contracted with local organizations to hire field staff and gather data. Data were requested from doctors and obtained from hospital records and death certificates. With approval of their doctors, a sample of cancer patients was interviewed. Local staffs did the preliminary editing and coding of data. Institute staff in Bethesda, Maryland completed the editing and coding of data and converted it to magnetic tape for use in preparing detailed reports through electronic computers.

Basic information was collected on every active cancer case, except certain skin cancers, during the three-year period. This included race, age, place and date of birth, sex, and marital status. Medical information included the primary site and histologic type of tumor, date and method of diagnosis. All reports were treated as strictly confidential. Death certificates mentioning cancer were obtained from the appropriate vital statistics offices. Local survey staff or hospital personnel abstracted information about cancer patients from hospital records.

Trained interviewers, after receiving written permission from attending doctors, obtained from a selected sample of patients information on the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions were also asked regarding religion, number of children, socioeconomic status, and personal habits such as smoking and drinking. Hospital records on this selected population were reviewed for more detailed information on cost of hospitalization, methods of meeting this cost, treatment for the cancer, and the extent of the disease at time of diagnosis.

The last batch of basic records was received in the central office from the fields in November, 1973. In January, 1974 a tape was created which contained data extracted into a single record for each patient from all basic documents ever received on a given patient, except that, if a patient had two or more cancers active during 1969-71, an extract record was created for each case. A similar extraction for sample data is discussed below. The last batch of sample documents was submitted to the central office in March, 1974. Patient interviews were submitted in July, 1974. These data have been extensively edited and extracts containing patient interview data, hospitalization financial data and hospitalization treatment data have been created.

A special skin cancer survey was started September 1, 1971 which collected data on cases diagnosed through February 29, 1972. Only four of the survey areas -- Iowa, Dallas, Minneapolis, and San Francisco -- were participating in this special study. Special efforts were made to contact all physicians who would see or treat skin cancer patients to obtain their cooperation. Pathology laboratory files supplied data on cases that were histologically diagnosed. In addition, abstractors completed separate forms for skin cancer patients found during their normal visits to participating hospitals. Data collection has been completed and magnetic tapes have been created to use in data analysis.

During the past year efforts have been concentrated on the analysis of various working files by individual investigators. An analysis of trends in cancer incidence and mortality 1935-1974 has been completed and submitted for publication. An analysis of cancer incidence by occupation and industry has been completed. Currently analyses of cancer incidence by marital status, socioeconomic status and urban rural residence are nearing completion and will be submitted.

Major Findings:

NCI Monograph 41 containing detailed incidence by race, sex and age of patient and by primary site and type of the cancer for each geographic area participating in the survey - except for Puerto Rico - and for all areas has received wide distribution within the United States. The monograph also has been distributed at two international meetings to participants from a wide range of foreign countries. Reaction to the monograph has been extremely favorable, especially from pathologists who for the first time have available to them incidence data cross classified by primary site and histologic type of cancer for all cancer sites and types.

Data were available on the patient interview epidemiology file for 7,518 patients with invasive malignancies. Comparison of the 7,518 interivew respondents with all TNCS patients for age, race, sex, marital status, method of diagnosis, vital status, country of birth and site distirbution showed only small differences and no strong nonrespondent bias. In the absence of normal controls a proportional morbidity analysis was employed.

Time trends in cancer incidence were analyzed by comparing data from seven areas which had participated in all three National Cancer surveys - 1937-8, 1947-8, 1969-71. The overall age-adjusted incidence rate for all cancer sites combined dropped 3.9% from 288.9 in 1947 to 277.7 in 1970. The drop was very striking among women: from 305.0 to 256.8 among whites and from 273.2 to 231.5 among nonwhites. Conversely, the rates for males increased from 283.7 to 309.0 among whites and went up 46.4% from 225.6 to 330.2 among nonwhites. Whereas the rates for females were greater than those for males in each race group in the earlier survey, the opposite was true in the more recent survey. The incidence rate for all sites combined was lowest among nonwhite males during the Second National Cancer Survey; in the Third National Cancer Survey, that population group experienced the highest incidence rate. Some explanations

for these diverse trends lie in the examination of the changes in rates for the individual primary sites.

Uterine cancer ranked number one in the first survey, and the rate has decreased 40% since that time. Breast cancer, which was tied with stomach for second place in the first survey, had the highest incidence rate in the second, even though it primarily affects only women. By the time of the third survey, the leader was lung cancer, having increased more than 350% since the first survey when it was the eighth most common primary site. Intestinal, prostatic, bladder, and pancreatic cnacer as well as the lymphomas have all shown increases, while stomach cancer has decreased a dramatic 72%. Rectal cancer has shown an overall decrease since the first survey, although there was an increase indicated in the second sruvey. These ten sites accounted for 73% of all cancer during the Third National Cancer Survey.

An in depth study of ovarian cancer was conducted to determine the relationships of histologic cell type, marital status, race and age. All types of ovarian cancer except germ cell tumors increased in incidence until the seventh and eighth decades of life, after which the rates plateaued. Epithelial tumors were equally common in young whites and blacks but were substantially more common in whites among middle-aged and older women. The incidence of nonepithelial ovarian tumors was similar in the two races. The incidence of ovarian cancer was 60-70% higher in women who had never married than in those who had. This relationship was present in both whites and blacks and in all age groups over 25 years. Among ovarian epithelial tumors, those for which the incidence rates between never-married and ever-married women differed most were endometrioid and clear cell tumors. The incidence of tumors of germ cell and sex cordmesenchyme origin, however, showed no relationship to marital status.

From the Interview Study, occupations and industries were tested for associations with specific cancer sites while controlling for age, sex, geographic location, education, and use of alcohol or cigarettes. Lung cancer patients were found more often than expected among several categories including trucking, air transportation, wholesaling, painting, building construction, building maintenance, and manufacturing (furniture, transportation equipment, and food products). Controlling for cigarette smoking did not change these associations. Leukemia and multiple myeloma were associated with sales personnel of both sexes, while lymphomas and Hodgkin's Disease showed an excess among women working in the medical industry. Other associations included rectal cancer with several retail industries; prostate with ministers, farmers, plumbers, and coal miners; malignant melanoma with school teachers; and invasive cervical cancer with women listed as working in hotels and restaurants. Breast cancer patients were more commonly found among women who were teachers or other professionals, and who worked in business and finance (even after controlling for education).

At the advice of the Third National Cancer Survey Utilization Committee a public use tape containing non-confidential data on the 181,027 incident resident cases of cancer has been available for purchase by medical researchers

at a cost of $100.

A public use tape containing data from the 7,518 successfully completed patient interviews has also been developed and made available for purchase at a cost of $100. Currently a public use tape containing hospital cost data is being developed. To date 15 universities and research groups have ordered the incidence public use tape and 6 research groups have ordered the patient interview tape.

Significance of the Program to the Institute:

This data base has already provided the Institute with the first reliable data on cancer incidence in the United States since the Second National Survey conducted in 1947. Data contained in Monograph 41 are a valuable source of information not available elsewhere in the country. Daily, a large number of queries are received on cancer sites and histology to which we are now able to respond with data. The information has also been useful in program planning and in preparing reports for NCI, NIH and HEW use. The survey data have also indicated there were errors in estimates of incidence by site used by various cancer agencies. The data will enable us also to provide a more sensitive method for determining estimates of the incidence of cancer in the future. Further, the availability of incidence data has enabled institute staff to assist other program areas, particularly the Cancer Control Program in site reviews and contract awards.

Economic data are now available for the first time in the history of the Institute. These data also will allow us to respond to data requests which come in daily regarding the economic impact of cancer. These data will be useful in program planning particularly in the area of cancer control and rehabilitation.

This data bank will provide a valuable source for epidemiologic investigations by identifying population groups that have a high or low incidence of cancers of specific sites. Some of the factors being studied include residence, occupation, place of birth, use of contraceptive devices, previous illnesses and socioeconomic status. Another area of special interest is rare tumors. Because of the low numbers of these tumors, epidemiologists feel that invesitgations will yield significant results and possible etiologic leads.

Proposed Course of the Project:

An analysis of cancer incidence by marital status is almost completed and will be available for publication. Analyses of cancer incidence among urban and rural residents of Iowa and Colorado are being readied for publication. Indicators for Spanish surnamed persons have been added to the incidence files and Spanish surnamed population files have been structured so that analyses of cancer incidence among this group can progress. These data will also be compared to incidence data from Puerto Rico.

The Laboratory of Pathology of NCI has conducted a feasibility study of patients whose cancer was confirmed at autopsy using TNCS data. This study was conducted in five hospitals in the metropolitan Atlanta area and results are being analyzed to determine whether a full scale effort should be launched.

Using census tract data socioeconomic indicators consisting of median family income, median education and percent of tract below poverty level have been added to the data file. Cancer cases have been grouped into five socioeconom categories specific for each race-sex group in each area. Current analyses are focusing on black/white differences controlling fro socioeconomic status. Other analyses are being planned and will be conducted during the coming year.

An analysis of cancer incidence among the foreign born population has almost been completed. A computer program is being developed to categorize cancer patients into ethnic origin groups based on surname so that cancer incidence among various ethnic groups can be studied.

Currently efforts are being concentrated on analysis of data from the financial portion of the patient interview. The data are much in demand, by the Division of Cancer Control and Rehabilitation who have supplied programming support for this analysis through contract with Abt Associates.

Incidence data being generated from the SEER Program for areas not included in the TNCS will be compared to look for differences in incidence among the various geographic areas.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04269-06-B

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biomedical Computer Systems - Consultation, Research and Development, Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Weiss	Head, ADP Management Section	BB, NCI
Other:	M. Cusano	Computer Programmer	BB, NCI
	A. Gales	Computer Programmer	BB, NCI
	D. Grauman	Systems Analyst, IPA	BB, NCI
	C. Hollingsworth	Computer Programmer	BB, NCI
	T. Hooven	Management Analyst	BB, NCI
	G. Jenkins	Computer Programmer	BB, NCI
	J. Larson	Supervisory Systems Analyst	BB, NCI
	M. Myer	Systems Analyst	BB, NCI
	A. Shevchenko-Mason	Computer Programmer	BB, NCI
	J. Stump	Supervisory Systems Analyst	BB, NCI
	V. Van Holten	Computer Specialist	BB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Biometry Branch, Field Studies & Statistics Program, DCCP

SECTION

Automatic Data Processing Management Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
18	8	10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to facilitate the research activities and other complex operations of the National Cancer Program through the application of computer science, information technology, management science and operations research; by minimizing the organizational, operational, mechanical and logistical constraints on scientific investigations and related activities which involve data and information collection, purification, storage, computation, retrieval, analysis and dissemination.

PHS-6040
(Rev. 10-76)

Project Description

The Automatic Data Processing Management Section provides consultation and assistance to the scientific and administrative staff of the National Cancer Institute, and as necessary, to other governmental agencies, private institutions, and individual investigators who collaborate with the National Cancer Institute in its mission. Consultation and assistance are given on individual, as well as on large-scale cooperative studies involving epidemiologic, laboratory and clinical investigations, cancer surveillance programs, cancer control programs, information and reporting systems for cancer centers, program planning and appraisal activities, and administrative management operations.

Methods Employed:

Collaboration, consultation, assistance, and research and development encompass four major areas of activity:

1. Planning, Design, Organization, Administration, Operation and Evaluation: Consulting and participating in the planning, design, organization and administration of programs and projects having data management and statistical computing requirements; establishing patient registries and statistical, data management and coordinating centers for large-scale cooperative studies; preparing reports on the technical feasibility and budgetary implications of various organizational, operational and methodological approaches to programs and projects utilizing computing equipment, and data management and statistical computing systems and libraries; conducting individual site reviews and participating on technical selection and review committee for grants and contracts; assisting investigators and contractors in identifying sources of, and in obtaining, statistical and computer-related resources and services; developing training programs and conducting seminars and workshops on various aspects of biomedical computing;

2. Development, Implementation and Operation of Data Management and Statistical Computing Systems and Libraries: Providing consultation and technical assitance through forms design, systems analysis, systems design, and the preparation and distribution of systems and operations manuals; providing computer programming services using digital computers, scientific computers with analog-digital converters, and related electronic instrumentation; providing generalized software systems and libraries for investigators' own use and to expedite and simplify the use of computers; conducting workshops and providing individual instruction in the use of specific computer systems, computer programs and equipment; coordinating and monitoring (a) the conversion of raw data into forms appropriate for computer processing, and (b) the day-to-day operation of data processing systems and related activities;

3. Research and Development in Biomedical Computer Systems and in the Organization and Management of Large-Scale Scientific Studies: Conducting research and development work in collaboration with computer scientists

and other specialists at universities and clinical centers throughout the country to develop new and improved methodology in the application of computers and management theory to biomedical research.

4. Operation of a Remote Terminal Facility: Operating and maintaining a Remote Terminal Facility with such equipment as the IBM 2922 Batch Entry/ High Speed Printer and Card Reader Terminal, and IBM 2741 Typewriter Terminals.

Major Findings:

ADP Management Section

For the past few years we have commented in our annual reports on the fact that we have received far more requests for consultation and assistance throughout the Institute than we have been able to provide. Furthermore, in spite of the heavy workload of each member of this Section, we have not been able to fulfill even the requirements of the SEER Program and our other FS&S responsibilities. We have utilized as extensively and effectively as possible a variety of mechanisms for obtaining additional resources, including an IPA position, consultants, assistance from the Division of Computer Research and Technology (DCRT), support contracts and purchase orders for consultation, systems analysis and computer programming. We have carefully reviewed the nature of the consultation and assistance that we have been providing and, given our limited resources, have established what we believe is a set of balanced priorities to meet the needs of the Field Studies & Statistics Program, other programs within our division, other divisions of the Institute, and the Office of the Director. On occasion, our expertise has been used by other Institutes to review and make recommendations concerning their programs.

As a result of the scope of our activities, we are in a position to see that the need in our own program area, and in other areas of the Institute, for biomedical computing policy, guidelines, consultation, review and evaluation, systems analysis and computer programming services, and for the development of special computer tools, is barely being met. The nature and extent of the work performed in this Institute, the type of organizational structure and other factors require that each program activity have its own independent, fully-dedicated biomedical computing experts, who can utilize, as the need arises, various additional sources of expertise as we have ourselves. It is not possible or appropriate for this Section and DCRT to attempt to provide all of the expertise and resources that are needed. This Section is, and should be considered, only a core resource for the Institute.

Not only are we not able to respond to the investigators who are aware of, and who desire assistance in applying computers to their research activities, but we see many areas where computers should be used, but are either not used at all, or are used in too simplistic a manner for the problems at hand. This under-utilization of computers stems from a variety of factors. They are: (1) a shortage of available computer experts; (2) the lack of familiarity with ways in which computers can assist investigators; (3) the cultural, historical,

and linguistical gulf between investigators and many computer experts; (4) the lack of funding support and reluctance to utilize positions for computer experts; (5) the lack of customized software tools designed for specific clinical, laboratory, epidemiological and other types of studies; and (6) the limited access investigators have to computers because of the amount of training and time required for them to use computers directly.

There are other consequences of the shortage of computer experts in the Institute. Over the past 10 years there has been a considerable expansion in the number of large-scale, long-term collaborative (multi-center) multi-million dollar programs and studies being conducted, as well as an expansion of the scope and complexity of these programs and studies.

Some examples and characteristics of these large-scale, long-term collaborative projects are:

1. Therapeutic and diagnostic clinical trials of long duration (three to five years or longer, with large study populations, including the screening of tens or hundreds of thousands of asymptomatic and symptomatic subjects, with participating institutions having distinctly different disciplines and responsibilities (e.g., to perform clinical examinations, laboratory and drug preparation and quality control, specimen testing, drug administration, special coding, behavioral studies, experimental and statistical design, analysis and quality control, patient registry operation and data management), relatively large numbers of professional and non-professional staff, a geographically dispersed multi-center network for data gathering and dissemination, a large volume of diverse events and variables (resulting in complex data bases and requiring sophisticated computer systems), extensive committee infrastructures requiring periodic national meetings, lay and scientific committee public relations, large complex budgets, and requiring the coordination of, and monitoring adherence to, uniform protocols and operational schedules;

2. Epidemiologic and demographic/descriptive studies, either retrospective or long-term prospective, with relatively large populations (of thousands to hundreds of thousands of subjects) which entail the gathering of large volumes of data from one-time or serially administered comprehensive questionnaires based on personal interviews, the abstracting of clinical, laboratory, and other data from medical records, death certificates, etc., the development and use

of intricate classification and coding schemes, the establishment and operation of central registries, statistical centers and computing facilities, and with relatively large numbers of professional and non-professional staff, the use of census data for geocoding and rate calculation, a network of geographically dispersed centers for data gathering, the long-term surveillance of selected cohorts of the population, a variety of working groups or committees having periodic national meetings, the adherence to operational schedules; and

3. Long-term education, prevention and demonstration programs, having relatively large populations (of thousands to hundreds of thousands of subjects), generally requiring extensive planning and management, the development of objectives and evaluation criteria, the development of implementation plans and adherence to operational schedules, the establishment and operation of patient registries and data reporting systems, the development of comprehensive computer data bases on the characteristics of the population (base-line data) using census data, data derived from the administration of questionnaires, and data derived from abstracting medical records, the coordination of lay and scientific community organizations, the use of public relations and media advertising, and the participation of relatively large numbers of professional and non-professional staff.

Additional computer experts are also needed in the Institute because of the complexity and extent of the comprehensive cancer centers program. Although some of the cancer centers have been established for many years and have fairly sophisticated computer facilities and staff, many of the new centers are facing extreme difficulty in determining what their computing resources should be, the type of equipment and staff they need, the appropriate location within the center for a computer core, the type of management information systems required, whether they should purchase generalized data base management systems or undertake development efforts of their own, etc. A clear and cohesive set of policies, guidelines, objectives and standards should be developed for this vital component of the cancer centers program.

In view of the shortage of positions, particularly the general reluctance throughout the Institute to use available positions for computer experts, it has been necessary to re-evaluate how we use the existing positions in this Section and any positions that we might obtain in the future. Rather than use our scarce positions for computer programmers who provide only computer programming services, it is more appropriate for us to utilize these same positions for senior biomedical computer specialists who can (1) make policy recommendations, and develop guidelines, objectives and standards, and (2) provide the broadest possible consultation to our division and program directors, branch and lab chiefs, grants administrators, project officers, and review and advisory committees, and to the scientific directors, principal investigators and others who collaborate with the Institute. These same senior computer specialists can establish

support contracts for systems analysis and computer programming services, utilizing more readily available contract funds.

The nature of the activities in the Section has also changed as a result of our efforts to compensate for the shortage of computer specialists and the extent of the computer work that needs to be performed. A fairly significant portion of our activities are now being devoted to developing generalized software packages designed for specific areas of clinical, laboratory, epidemiological and other types of research, to allow investigators and their staff to operate as independently as possible and without the need for as much assistance from systems analysts and computer programmers. Our modus operandi is also designed to establish, in each program area with which we consult, their own independent software development capabilities. Our consultation includes: (1) familiarizing their investigators with ways in which the computer can assist them in their research; (2) developing one or more generalized computer systems for use by their investigators, lab technicians, nurses, etc.; (3) maintaining if necessary, the generalized computer system over time; (4) using funds provided by that program area for data management and statistical computing support services contracts; (5) preparing an RFP for, and assisting the program area, in obtaining a support-services contract for systems analysis, computer programming, data entry and system operation; (6) advising the division director, branch or lab chief, section head, committee chairperson, etc., of the need and justification for their own biomedical computer specilist(s), computer technician(s), etc.; and (7) assisting in the recruitment and orientation of computer specialist(s), technician(s), etc.

Another facet of our planning to alleviate the problems stemming from the shortage of computer experts in the Institute and the under-utilization of computers, involves establishing a Computer Systems Branch within the Institute. This year we have begun developing a proposal to establish a Computer Systems Branch within the Field Studies & Statistics Program. A portion of this proposal will include an analysis of the Institute's current biomedical computing activities, resources and services, and an analysis of the utilization of computers, a justification for establishing a Computer Systems Branch, a proposed organization structure, functional charts, staffing charts, application charts, and recommended budget. This will be done for both the short-term and long-range needs of the Institute. However, because of our many commitments, progress on this proposal has been very slow.

The following report on this Section's activities reflects the changes in our mission, objectives, assignment of staff, the type of expertise we require, and the technical approaches that we have considered to be necessary in order to meet the needs of the Institute over the next few years.

Office of the Director, NCI

Frederick Cancer Research Center, EDP Working Group

Mr. Larson has been a member of the FCRC EDP Working Group for three years. This group is comprised of consultants in the computer field

from the Office of the Director, NCI, from the Viral Oncology and Chemical Carcinogenesis Program of the DCCP, and from the Division of Computer Research and Technology (DCRT). The group is responsible for monitoring all aspects of the data processing portion of the Litton Bionetics FCRC contract. This involves monthly reviews of technical work in progress and an annual review (at contract renewal time) of the accomplishments, plans, problems and cost of the contract, which includes a data processing staff of approximately twenty computer programmers, systems analysts and operations specialists who provide support services to the FCRC. Application areas include administrative management systems, animal inventory management systems and laboratory equipment monitoring systems. Mr. Larson's consultation at the FCRC is expected to continue at the same level.

Systems Planning Branch

Mr. Weiss is a consultant on the development of the National Cancer Institute's Management Information System through membership and participation in the MIS Configuration Management Committee. This group is responsible for reviewing, coordinating and advising on technical aspects of the various subsystems in the MIS.

Division of Cancer Cause and Prevention

The Field Studies and Statistics Program

The Section's workload in the Field Studies and Statistics Program has continued to expand this year with our involvement in new programs and studies and our increased participation in the Surveillance, Epidemiology and End Results Program. In response to this increasing workload, we have raised the level of effort on our computer programming support services contract with GEOMET, Incorporated, to eighteen person-years (versus six last year) and have continued to assign the majority of our staff to this area. Mr. Larson, Ms. Van Holten, Ms. Cusano, Ms. Wolfson and Mr. Hollingsworth are working full-time on FS&S activities. Other ADPMS staff are involved on a part-time basis as their specific expertise is needed.

Office of the Associate Director for Field Studies and Statistics

Ms. Cusano and Ms. Parsons developed a computer system to keep track of the publications and reference books maintained throughout the FS&S area, and to monitor subscriptions to periodicals and books on order. This involved designing and programming a computer system, which uses the WYLBUR text-editing system and the IBM 2741 terminal, establishing a database of all FS&S reference materials, and training secretarial staff to operate the system.

The Surveillance, Epidemiology and End Results Program

The SEER Program is the major focus of the Section's consultation and support activities. The nature and level of our participation in SEER has changed considerably this year, with an increased emphasis on participation in the overall direction and operation of the Program and on consultation with participating registries in upgrading their management capabilities and improving their organizational structures and staffing plans. Ten staff members devoted approximately seven person-years of support to the SEER Program.

1. Participation in Program Direction and Operation

All three of the ADPMS's senior staff are members of the in-house committees which direct and conduct the SEER Program. Mr. Weiss is on the SEER Program Review and Analysis Committee (SPRAC) and the SEER Coordinating Committee; Mr. Stump in on the SEER Data Analysis Committee; and Mr. Larson is on the SEER Data Acquisition Committee and the Operations Committee of the SEER Executive Group. Additionally, Ms. Cusano served as a consultant to both the Data Acquisition and the Data Analysis Committees.

Ms. Cusano worked closely with the Data Acquisition Committee, the Data Analysis Committee, the Field Liaison Section, the SEER project officers, and Ms. Paulette White of the End Results Section to plan, direct, and coordinate the 1977 SEER data submission. Ms. Cusano and Ms. White assumed full responsibility for, and carried out, documenting submission requirements, receiving and processing computer tapes from the registries, coordinating the error correction process, and generating recoded files and tabulations for invesigation, publication and evaluation purposes.

Ms. Cusano authored a section of the SEER Program Manual describing NCI's handling of the 1977 data submission, specifying the current reporting requirements, and detailing plans for the 1978 processing cycle.

Operational problems in the Iowa Tumor Registry required that we develop a special computer system to perform all of the data preparation functions normally completed in the field for the 1977 SEER submission. Mr. Larson and Ms. Cusano planned and organized this project and directed the activities of a consultant, Mr. Norman Oslik, and a GEOMET, Incorporated computer programmer, for the three-month period required to complete the project.

2. Organization and Management of SEER Registries

Last year the Section initiated a series of workshops to upgrade the organization and management capabilities and to improve the overall operating effectiveness of the eleven population-based tumor registries participating in the SEER Program.

Three workshops for registry administrative directors were conducted this year by the Section. The objectives of the first workshop were to: (1) introduce registry directors and NCI SEER staff to general management concepts; (2) determine the organizational structure, staffing and management approaches currently used in the registries; (3) identify problems in the current organizational and operational approaches; and (4) develop an outline for a SEER registry model organization structure. In the second workshop, Mr. Louis Voegler, Chief of the Executive Development Branch, NIH, presented a three-day seminar on management concepts and practices. This seminar was prepared by Mr. Voegler specifically to address the administrative and management problems of large-scale, long-term operations in a biomedical research environment. A portion of the workshop was devoted to presentations by registry administrative directors of successful solutions to management problems which they had implemented in their organizations. The third workshop focused on the further development of a model organizational structure for a SEER registry. The completed model will be published in the SEER Program Manual, and will include sample organization, staffing and functional charts, position descriptions, etc. This will be used in the organization of new registries and in the evaluation and upgrading of existing ones. This project is expected to continue next year with additional workshops and seminars for SEER registry administrative directors.

3. Reorganization of Reporting Systems in the California and Connecticut Tumor Registries

Mr. Larson and Ms. Van Holten continued to direct, coordinate and participate in the cooperative effort between the California and Connecticut Tumor Registries to reorganize and upgrade their reporting systems. This effort, which has been underway since early 1976, involves integrating the design, and sharing the development of, a uniform data management, reporting and statistical computing system for large population-based tumor registries.

Although the system, with some adaptions, will be implemented independently, in each of the two registries, the fact that this is a joint venture has established a precedent in cooperative studies of this nature within the SEER Program and provides significant benefits to the Institute, which would not have been realized otherwise. The most important of these benefits to the NCI is the transportability of components of the system to other SEER participants and to other NCI programs with population-based reporting requirements.

Mr. Larson has continued to serve as the project director this year. He conducted two site visits to each registry to review progress and to resolve problems; presented three progress reports to the Biometry and Epidemiology Contract Review Committee and two reports to the SEER Executive Group; delivered seminars to the Iowa, New Mexico, and Detroit SEER registries explaining the concepts employed in the system; and organized a computer programming team in Bethesda to complete the system.

Ms. Van Holten spent four months (September - December 1976) in Berkeley, California to complete computer program specifications for the system and she made ten visits to Connecticut and California to coordinate technical work. Since that time she has been responsible for the technical direction of four computer programmers who are under contract and two ADPMS staff programmers who are working on this project in Bethesda.

Mr. Weiss, Mr. Stump, Mr. Larson, and Ms. Van Holten conducted three workshops in Bethesda to evaluate detailed implementation plans, budgets, and schedules, and to critique work completed during the year.

Progress on this project has been slower than projected due largely to the technical, administrative, project management, and staffing problems associated with a complex, geographically dispersed, cooperative effort of this type. These delays have necessitated a revised approach to systems development and implementation in order to accommodate operational requirements of the registries and the SEER Program. Essentially, these changes involve: (1) a centralization of the majority of the computer programming effort in Bethesda to improve technical project management; and (2) a phased implementation with a 'core' system installed first in California and subsequently expanded for installation in Connecticut.

The 'core' system will be programmed by the end of 1977 and will be operational in the California Tumor Registry in February 1978. It will incorporate the essential elements of the complete system, such as revised file and code structures, automated patient linkage, new input forms and operational reports, and computer-assisted tumor record consolidation. Many design features such as the automated, between-record consolidation of therapy data will be developed after the initial implementation of the core system. These capabilities will be included in the complete system to be installed in Connecticut in January 1979. This approach is necessary for California to meet its January 1978 SEER submission deadline.

Progress this year includes: (1) the completion of computer program specifications for the entire computer portion of the data management system and computer programs for the California Tumor Registry's core system; (2) the conversion of existing files and records in the California Registry; and (3) the development of revised organization, staffing and functional charts and position descriptions for both registries.

Plans for next year are to: (1) expand California's core system for use in Connecticut; (2) implement revised organization structures and staffing plans in both registries, including securing State civil service approval; (3) adapt systems components for use in other SEER registries; (4) begin computer programming of the statistical and analytical components of the system; and (5) study the feasability of transplanting the system's concepts and components in other NCI programs.

4. Report to the Biometry & Epidemiology Contract Review Committee

In response to a variety of questions that arose concurrently from (1) a site visit of the Connecticut Tumor Registry and the Connecticut Cancer Epidemiology Unit, (2) a request for a relatively large amount of funds by the Yale Comprehensive Cancer Center to develop a data reporting system, and (3) concern over the delays and increased cost of the joint Connecticut/California data management system, Mr. Weiss presented an informal paper to the Biometry & Epidemiology Contract Review Committee on the subject of NCI coordination of, and the cost of, data reporting systems. Although several members of the Biometry Branch have consulted with the cancer centers program, specifically in the establishment of their reporting system (CCPDS), and in the cancer control program, which includes the establishment of population-based registries, there is a need for more extensive coordination and consultation. In order to provide this consultation, the Director, NCI, has established a coordinating committee of representatives from the various NCI programs involved in the development of large data reporting systems. As we have observed in other portions of this report, the serious shortage in the Institute of computer experts with experience and expertise in biomedical data reporting systems will continue to make it difficult to coordinate these programs and to avoid expensive duplication of effort. In our estimation, because of the heavy workloads, and without additional professional staff, it will be very difficult for the Biometry Branch to provide the consultation and coordination required.

The delays in completing the new population-based registry data management system, developed initially to replace the present systems in Connecticut and California, and most likely to be used in Iowa, Atlanta, and possibly Detroit, have resulted primarily from administrative problems in California, which delayed their full participation in the project for approximately one year, the difficulty in obtaining computer experts in these areas to carry out the project, the additional time and cost of developing a system that is transportable in whole or in part to most of the population based registries in the SEER Program, and the difficulty in integrating and negotiating the different design concepts and specific needs of each registry into one system in which there are common approaches to handling complex functions. This latter aspect of the new SEER data management system project has been the single most difficult and time-consuming part of the project, and designing and building transportability into the system has been no small technical accomplishment. Furthermore, it is very difficult to evaluate the cost and time of developing this system because there are relatively few others of the scope and complexity to compare it to. We think that the benefits that will be derived from the common approaches and transportability will have far reaching implications for cancer reporting systems throughout the United States for many years to come.

A final point in this report to the Biometry & Epidemiology Contract Review Committee is that large-scale computerized data management systems are now a field of expertise all their own, and are no longer a subspecialty of biostatistics.

5. The Iowa Cancer Epidemiology Research Center

For the past several years, the ICERC has experienced a number of serious problems, including staff turnover, lack of experienced data processing personnel, incomplete systems and procedures, and a variety of other organizational, operational and administrative problems. This culminated in their failure to meet their 1976 SEER submission deadline. Mr. Weiss, Mr. Stump, and Mr. Larson, at the request of the Principal Investigator, conducted an extensive analysis of the ICERC.

An outline of this review is included here in order to indicate the scope and depth of the managment analysis and systems analysis, which this Section provides as a consulting service to our population-based registries. Our analysis covered the following areas:

(a) The background and history of cancer registration and reporting in the State, and of the Iowa Cancer Epidemiology Research Center;

(b) The characteristics of the State which have implications on registry organization, management, administration, workloads, staffing and costs (e.g., population size, distribution, and characteristics, geography, legal basis for reporting cancer, referral practices and patterns, attitudes of medical and community organizations toward cancer reporting);

(c) The ICERC's relationships with the University, the College of Medicine, the Department of Preventive Medicine and Environmental Health, the personnel and business offices of the University and the Administrative Office of the College of Medicine;

(d) The present status and problems associated with the 1977 SEER submission, including the ICERC's organization (e.g., structure, functions, staffing, facilities), management (e.g., objectives, priorities, internal coordination, supervision), workloads, budget, data systems, computer center, operational procedures, documentation, and schedules.

At the conclusion of this site visit, a detailed report was prepared including short-term and long-range recommendations for resolving the problems uncovered in the study. This type of analysis has now been conducted in four of the largest registries in the SEER Program. The nature of the problems experienced in these large-scale operations are similar to some extent. It appears that there is a need for:

(a) More clearly defined and clearly interpreted SEER Program and individual participant policy, goals, objectives, priorities and plans;

(b) A more realistic assessment of the complexity of establishing concurrently, a population-based registry and a center for epidemiologic research and training;

(c) The implementation of a SEER-wide program management information and cost reporting system; and

(d) More extensive NCI advice and assistance in establishing, reorganizing and upgrading SEER participants, such as the ICERC.

Several additional site visits were conducted to provide assistance in the implementation of our recommendations and to monitor their progress.

As part of our effort to assist the Iowa Cancer Epidemiology Research Center in its reorganization and upgrading, we conducted a three-day workshop in Bethesda for the registry's chief coordinator, Mr. Ralph Kessel and the registry's senior systems analyst, Mr. Kevin Gleaves. The workshop included: (1) reviewing the registry's problems in meeting its 1977 SEER data submission requirements; (2) analyzing in detail every computer program in their data management system; (3) turning over to Mr. Gleaves the computer programs designed by our Section and programmed by GEOMET, Incorporated, to extract, consolidate, and edit cases from the Iowa master file to create their 1977 SEER tapes; (4) discussing the basic concepts of population-based tumor registry data management; and (5) describing what we consider to be the advanced techniques that we have developed over the past ten years, particularly those techniques developed in the joint Connecticut/California data management system project.

Our consultation with the ICERC has resulted in the implementation of all of the recommendations in our site visit reports. These recommendations included a revised organizational structure, a new staffing plan, the upgrading of several key positions, the establishment of several new positions, the rewriting of position descriptions, the preparation of a more realistic budget, the establishment of closer coordination and liaison with the administrative and personnel offices, the assignment of several computer specialists from the University's computer center to the ICERC, the expansion of the ICERC's facilities and the consolidation of quarters, which had been located in several buildings.

6. The New Orleans Tumor Registry

At the request of the director of the New Orleans Tumor Registry, Mr. Stump consulted with her on the selection of a new computer center, reviewed the registry's data base management system and plans for further system development, and assessed the registry's computer-related staffing requirements.

Mr. Stump visited four computer centers in New Orleans, and based on his evaluation of their hardware, software, cost, reliability, and reputation, recommended the use of the Tulane University Computer Center. He also recommended several changes in the registry's computer programs, system development plans and approaches, and computer-related staffing. This included: (1) streamlining error handling procedures; (2) developing document control checks; (3) implementing automated patient linkage routines; (4) adding a systems analyst to the registry staff; and (5) installing a remote terminal with a print capability in the registry to facilitate computer program development and data analysis. These recommendations were accepted by the registry director. Mr. Stump will continue to work closely with the New Orleans Tumor Registry staff as these recommendations are implemented.

7. The Atlanta Tumor Registry

The Atlanta Tumor Registry is the latest addition to the SEER Program. During the past two years the registry has been involved in planning, developing and implementing its data mangement system. Unfortunately, it has encountered many of the same difficulties that other registries have experienced in meeting this complex objective. At the request of the project officer, the senior members of this Section met in Bethesda with the Atlanta Tumor Registry's director and the registry's computer specialist to review technical approaches, progress and problems related to the development of their data management system.

As a result of this meeting, we recommended a set of short-range and long-term objectives, revised technical approaches and staffing changes. The new data mangement system under development for the California and Connecticut Tumor Registries will provide procedures, computer subroutines and other material for use in Atlanta, when this material becomes available. However, an interim system will have to be constructed if the registry is to meet its current submission deadline. This Section has continued to consult actively with the Atlanta Tumor Registry to assist with the implementation of our recommendations and to monitor their progress.

8. SEER Cost Analysis

As with any large-scale, long-term, multi-center program, it is very difficult to determine the cost-effectiveness of the overall operation of the SEER Program, and its individual components. This is particularly true when a large portion of an operation involves activities that are difficult to quantify (e.g., quality control, data management, statistical computing). The problem of cost analysis is further compounded by the diversity of legal, organizational, geographic, economic, local reporting requirements and other factors. Our review

and advisory committees, project officers and in-house staff have struggled with this problem since the inception of the SEER Program, during the Third National Cancer Survey, and over the past 20 years in the End Results Program. The establishment of new population-based registries under the Community-Based Cancer Control Program, and the possibility of adding other registries to the SEER Program, has raised this issue to a very high level of priority.

Several steps have been taken in order to come to grips with this problem. A SEER Program Review and Analysis Committee has been established within the Biometry Branch to focus on this and other key problems. Mr. Weiss and Mr. Tom Hooven, in collaboration with Mr. William Lourie, End Results Section, have begun an analysis of the structure, functions, cost centers, cost variations and cost-contributing factors of the registries within the SEER Program. Some analysis of costs within a single registry has been done successfully by the New Mexico Tumor Registry, and they have been quite helpful in providing advice and material for our own analysis. We believe that the information developed by this kind of analysis will be useful in developing a better understanding of the cost of the program, lead to the identification of under-funding and over-funding situations, if any, and possibly result in the development of one or more organizational and budgetary models, guidelines, evaluation procedures, etc., which can assist project officers, site visitors, principal investigators, and others in planning and reviewing these large operations. On the basis of our preliminary studies, it appears that it is not possible to develop an algorithm that can be applied to each registry to accurately measure the cost-effectiveness of the registry. This is not possible because of the variety of conditions and local variations affecting the structure and function of each registry.

International Death Certificate Study

During this year, Mr. Michael Stump collaborated with Ms. Constance Percy and staff members of the Office of Health Statistics, and concluded work on the International Death Certificate Study. The study is designed to determine the variations in the cause of death coding between the United States, Canada, Great Britain, Norway, France, the USSR and the Federal Republic of Germany. Approximately 1,250 mortality cases were randomly selected from the Third National Cancer Survey data base and photostatic copies of the death certificates corresponding to these cases were forwarded to health ministries in each of the participating countries responsible for cause of death coding. Computer programs were written by staff programmers under Mr. Stump's direction to edit the data returned from each country and to compare ICD codes at both the three-digit and four-digit levels.

A computer program was designed and programmed to produce camera-ready copy for publication of the final report. The production of the final camera-ready tabulation effectively concludes our participation in this project.

International Classification of Disease for Oncology (ICDO)

Ms. Shevchenko-Mason is the principal consultant to Ms. Constance Percy and to representatives of the World Health Organization in a project to develop a cancer supplement for the 9th Revision of the International Classification of Disease for Oncology. A field trial conducted to determine the adequacy of ICDO in describing neoplasms has been completed during the year. Data on approximately 3,500 test cases have been received from tumor registries in the United States and throughout the world. Computer routines have been developed by Ms. Shevchenko-Mason to edit, store and analyze the field trial data.

Additional data were received from the participants in the field trial and were included in the analysis of the data already collected. Complete reports were generated to tabulate the frequency of use, for each individual morphological and topographical code, and for the problems encountered in the use of this code. These tabulations were used by the World Health Organization to review and to refine the code structure. The World Health Organization has now published the ICDO code and future ADPMS involvement in this area will be focused on coordinating the conversion of files maintained in SEER registries and by NCI investigators.

Therapy of Patients with Malignant Melanomas

Ms. Darlene Myer and Ms. Ruth Wolfson have been consulting with Dr. Bernard Fox on a study of the psychosocial and psychobiological characteristics of patients with malignant melanomas. The objective of the study is to determine the role of psychosocial factors in the progression of malignant melanoma from clinical stage II to stage III. During this year, Ms. Myer designed and implemented a computer system to collect, edit and store the patient data and Ms. Ruth Wolfson wrote a computer program to format and list these data. Several series of data analyses focusing on correlations and multiple regressions were run using SAS and BMD routines. The data management system and the library of statistical routines have been turned over to Dr. Fox's research assistant who is responsible for all of the operational aspects of this study. Our consultation on this project is expected to continue on an ad hoc basis.

Third National Cancer Survey

The Third National Cancer Survey (TNCS) was this Section's first major consulting and support responsibility. This support required a majority of our staff and contract resources from the early planning phase of the study in the fall of 1967, through the completion of the data gathering and data purification phase in the summer of 1974.

This year our direct involvement in this project has decreased significantly. Many of the computing requirements of in-house TNCS studies are now routinely handled by investigators using generalized software packages and data files designed and prepared by the ADPMS for this purpose. Mr. Larson and Mr. Hollingsworth completed a project to convert the survey's one million abstracts to microfiche storage, to improve confidentiality and security, and to conserve office space. GEOMET, Incorporated provided eight person-years of systems analysis, consultation, and computer programming service to twenty TNCS-related studies and operational projects. Next year the contractor's level of effort in this area is expected to decrease to approximately five person-years.

End Results in Cancer

Ms. Mary Cusano is the principal ADPMS consultant to the End Results Section of the Biometry Branch. This year she participated in the publication of End Results in Cancer Report No. 5, as a follow-on to her extensive data preparation work in this area last year. This joint effort produced finished type-set output for hundreds of graphs and tables, which were then converted into camera-ready copy. In contrast to previous methods requiring hand typesetting and proofing, this technique used the generalized software system TPL (modified by the Bureau of Labor Statistics for this project), to produce direct input files to the Government Printing Office's computer-driven typesetting equipment. This methodology will result in a more rapid and less costly production of future publications. In addition to designing and writing numerous computer programs, Ms. Cusano was responsible for coordinating the computer-related work of End Results Section investigators, statisticians and computer programmers, a commercial artist, the NIH publications office, the Government Printing Office and the Bureau of Labor Statistics.

Incidence of Non-Melanotic Skin Cancer and UV Radiation Exposure Study

Mr. Larson consulted with Mr. Scotto and Dr. Fears on the organization and operation of a joint EPA/NCI twelve-month study to measure the incidence of non-melanotic skin cancer and the level of ultra violet radiation in seven geographic areas of the United States. Mr. Larson designed the computer processing system, forms and methodology needed to collect and process incidence and UV data. He participated in negotiations and technical meetings with the registries, and organized and monitored a support contract with GEOMET, Incorporated, to conduct the data collection and purification phases of the study, and to provide computer programming services to Biometry Branch investigators.

The Environmental Studies Section

Funds were added this year to the GEOMET, Incorporated, support contract to provide three person-years of computer programming and systems analysis services, on a trial basis, to the Environmental Studies Section, Environmental

Epidemiology Branch. Mr. Larson worked with Dr. Mason and Dr. Hoover to determine their data management and statistical computing requirements and to modify the contract workscope. Mr. Larson coordinated the phasing in of additional contractor staff and worked with the contractor to implement an accounting system to monitor charges by project. GEOMET's performance in support of Environmental Epidemiology Branch investigators has proven to be quite effective. Plans are to gradually increase the level of effort each year as the Environmental Epidemiology Branch's staff and programs grow. Projects involving GEOMET this year include: (1) The Effect of Industrial Cutting Oils on Industrial Workers; (2) Reserpine and Breast Cancer; (3) Site-Specific Cancer Mortality Studies; (4) Water Quality Sampling and Correlation with Cancer Mortality; and (5) Cancer Mortality and exposure to Dry Cleaning Fluids.

Division of Cancer Biology and Diagnosis

The ADP Management Section provided consultation and assistance on the following research projects conducted by the Division of Cancer Biology and Diagnosis.

Immunology Branch

The clinical research program of the Immunology Branch is primarily involved with immunobiology laboratory investigations, with particular emphasis given to studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response system, and transplantation.

Under Mr. Stump's direction, Ms. Darlene Myer and Ms. Anya Shevchenko-Mason are implementing a data management, retrieval and analysis system specifically designed to remove some of the restraints to utilizing the computer that have traditionally existed within the clinical research environment. The system is designed to allow investigators to retrieve specified clinical and laboratory test results and to display these values for a specified patient, for a selected subset of patients, or for the entire patient population under study. With minor modifications to the record description section, the system can be used to retrieve data from any patient study data base. The system is being implemented and tested on a small-to medium-size clinical trial, that is being conducted to evaluate several courses of therapy for patients diagnosed with malignant melanoma.

Major system features include:

1. A capability to combine data from several different sources (e.g., laboratory test records, patient admission records, patient work-up records);

2. A prompting feature which allows investigators to input parameters that specify patient selection criteria, the laboratory test requested, the time intervals between examinations, and the particular graph or report format desired.

3. A capability to generate automatically a limited amount of statistical calculations.

4. A choice of two different report formats.

This clinical and laboratory research tool was conceived of, and designed by, Mr. Stump and Ms. Myer in consultation with Dr. John Wunderlich of the Immunology Branch. Mr. Stump prepared the RFP to obtain the services of a commercial data processing contractor. The commercial contractor, Ebon Research Associates, has the responsibility of finalizing the detail design of the system, for doing the computer programming, and for documenting the system. Mr. Stump is currently serving as the co-project officer on this contract. Technical monitoring and acceptance-testing are being coordinated by Ms. Myer and Ms. Shevchenko-Mason.

Future system development plans include increasing the statistical capabilities currently available to the user, and adding new optional output report formats. A system demonstration is being planned for the DCBD branch and lab chiefs. We feel that our continued participation in this project offers us an opportunity to contribute to the solution of some of the data management and analysis problems that exist not only in the research program of the Immunology Branch, but also in the research being conducted in other branches and labs within the DCBD. We plan to modify the system and to make it available to these other research programs upon request.

Committee on Immunodiagnosis

The Committee on Immunodiagnosis is involved primarily in the review and funding of research contracts associated with studies of cell-mediated immunity to tumor associated antigens, and in the development of new procedures for the immunodiagnosis of cancer. Dr. Ronald Herberman, Chief of the Laboratory of Immunodiagnosis, is Chairman of the Committee. Committee members are selected from other research institutions throughout the United States. Mr. Stump and Dr. Mitchell Gail, Clinical and Diagnostic Trials Section, DCCP, continue to consult with the Committee on the analysis of results from immunodiagnostic tests and to review the data management, statistical computing and general recordkeeping components of research contract proposals received by the Committee.

A year ago, Dr. Herberman requested that the ADPMS participate in solving the data management and statistical computing problems associated with a program of research, where investigators request a panel of sera from

NCI to test the sensitivity and specificity of new diagnostic procedures. In response to this request, Mr. Stump and Ms. Myer conducted a requirements analysis study to: (1) become familiar with the work of the Committee; (2) determine the most appropriate data management system which could be used to collect, edit and store the data; and (3) identify the sources of statistical analysis routines, which could be used to evaluate the effectiveness of the various tests. Following this study, we designed a data collection form for recording pertinent test information, and designed and implemented a data management system. In consultation with Dr. Gail, we also compiled a library of statistical and graphical routines to facilitate the evaluation of the effectiveness of the immunodiagnostic tests. To date, we have performed the analysis on approximately forty-five tests.

Mr. Stump and Ms. Altamease Gales have also developed an RFP to secure the data processing services that are necessary to continue this level of support to the Committee on Immunodiagnosis. The data management system developed by the ADPMS and the library of statistical routines that we compiled will be turned over to the commercial vendor. Ms. Gales will serve as the technical liaison to the contractor. Dr. Gail will also continue to provide statistical consultation.

Plans for future ADPMS consultation to the Committee include responding to requests for assistance in the automation of the Mayo Clinic Serum Bank and for consultation on the data management aspects of a clinical and laboratory study being conducted by the University of Minnesota, that have been deferred due to other priorities.

Division of Cancer Research Resources and Centers

Cancer Centers Program

ADPMS consultation with the Cancer Centers Program has increased during the past year in response to requests for assistance from Dr. Genrose Copley, director, Cancer Centers Program, Mr. Richard Sherbert, program coordinator for the Association of American Cancer Institutes (AACI) and Mr. Thomas Dundon, project officer for the Statistical Analysis and Quality Control (SAQC) Coordinating Center contract. The Cancer Centers Program has been established to develop multi-disciplinary cancer centers, which can engage in clinical and basic research and influence the upgrading of cancer care in surrounding communities.

Mr. Weiss, AACI/NCI Chairman of the Subcommittee on Data Processing, was asked to review and to evaluate the data processing component of the AACI contract proposal. Other ADPMS staff participating in this review included Mr. Larson, Ms. Myer, Mr. Stump and Ms. Van Holten. The contract proposal called for the development of: (1) a survey report describing selected centers; (2) ADP guidelines for center directors; (3) a report to NCI

assessing data processing functions in centers; and (4) criteria for use by NCI in evaluating data processing aspects of grant and contract proposals. The ad hoc review committee concluded that the proposal was incomplete, lacked detail and reflected deficiencies in understanding the problem. On the strength of the subcommittee's findings, the data processing component of the AACI contract was withdrawn subject to review and subsequent resubmission on the basis of a collaborative effort between ADPMS staff and the AACI to develop a better understanding of the problem and to design a more appropriate approach to solving it. The issue of the effective and efficient use of the computer is vital to the research programs and clinical activities of the cancer centers. However, because of the shortage in professional staff in the ADPMS, we have not yet been able to respond to this request for additional assistance.

During this year, Mr. Michael Stump also served on the contract review committee which was responsible for approving funding to staff the Epidemiology and Statistical Units being organized in each cancer center. Evaluations focused on proposed computer budgets, core staffing requirements and the methodology proposed to accommodate the reporting requirements of each center.

Mr. Stump also served as the government consultant during negotiations for the contract to select a Statistical Analysis and Quality Control (SAQC) Coordinating Center. In this capacity, he was responsible for evaluating the two most responsive proposals in terms of the organization and management of the project, the operating budgets, the hardware/software configuration and the overall technical approach. A final recommendation was made to award the contract to the Fred Hutchinson Center in Seattle, Washington. Mr. Stump will continue to consult with the project officer on the data management and statistical computing aspects of the Coordinating Center.

Division of Cancer Control & Rehabilitation

Diagnosis Branch, DCBD

Breast Cancer Detection Demonstration Program

The ADP Management Section has phased out of its active role in this joint National Cancer Institute-American Cancer Society Project. Since 1973 we have participated in the planning, coordination, and direction of this project, particularly in the data management and statistical computing components. The scope of this enormous program required far more than the 1-1/2 staff members this Section was able to devote to this project's data management and statistical computing areas. As of the end of the last reporting period, the Division of Cancer Control and Rehabilitation was able to assume full operational responsibility for this program and assigned their own staff members in lieu of this Section's systems analysts. Because of the extensiveness of our earlier role, we had a

considerable amount of documentation, files, schedules, and on-going activities with individuals from all of the 30-odd institutions and organizations participating in this project. In order to provide as smooth a transition as possible, we conducted a number of orientation meetings with DCCR staff, provided them with a detailed work plan of all on-going and planned activities and shipped all of our documentation, administrative and technical files to the DCCR staff. Our present role has been to provide occasional consultation on various aspects of the program.

Prevention Branch

Tyler Texas Asbestos Workers Program

As with the Breast Cancer Detection Demonstration Program, the ADP Management Section has phased out its active role in this program. Mr. Weiss has been serving as a consultant on this project since 1975 and played a major role in establishing their Statistical/Coordinating Center. As a result of DCCR's ability to provide its own data management expertise on this project, we were able to reduce our involvement to one of minimal consultation.

NIH Collaborative Activities

LARSON, J.: Member, Contract Review Committee, National Eye Institute, Coordinating Center for the Diabetic Retinopathy Study, University of Maryland, Baltimore, MD.

LARSON, J.: Consultant, Clinical Research Branch, Division of Extramural Research Programs, NIMH, Collaborative Program on the Psychobiology of Depression.

LARSON, J.: Consultant, National Center Health Services Resources, USPHS, DHEW, Medical Care Expenditure Survey.

STUMP, J. M.: Consultant, Transplantation Branch, NIAID, Kidney Transplant Histocompatibility Study, Statistical Center, Naval Research Biological Laboratories, Bethesda Naval Medical Center.

WEISS, T.: Member, Grant Application Review Committee, Division of Extramural Affairs, NHLBI, Baylor University College of Medicine, National Research and Demonstration Center.

WEISS, T.: Member, Contract Review Committee, Division of Research Resources, NIH, CLINFO Research Project, University of Washington, College of Medicine, General Clinical Research Center.

WEISS, T.: Member, Contract Review Committee, Division of Research Resources, NIH, CLINFO Research Project, Vanderbilt University, College of Medicine, General Clinical Research Center.

WEISS, T.: Chairman, Contract Review Committee, Division of Heart and Vascular Diseases, NHLBI, Central Coordinating Center for Lipid Research Studies, Department of Biostatistics, University of North Carolina.

WEISS, T.: Member, Steering Committee, Development of a Coordinating Center Model for NHLBI Clinical Trials, Division of Heart and Vascular Diseases, NHLBI, University of Maryland.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04409-02 B

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Statistical Methodology Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. P. Byar	Head, Clin. & Diag. Trials Section	BB	NCI
OTHER:	M. H. Gail	Medical Statistical Researcher	BB	NCI
	S. B. Green	Medical Researcher	BB	NCI
	J. A. Koziol	Staff Fellow	BB	NCI
	L. R. Muenz	Mathematical Statistician	BB	NCI
	D. K. Corle	Computer Systems Analyst	BB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch, FS&S Program, DCCP

SECTION
Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research in statistical methods with particular emphasis on those appropriate to the analysis of data from clinical and diagnostic trials and epidemiological studies of cancer. Many of the problems studied under this project arise from the consultative activities of the Section. In the past year this research has included a study of methods of comparing multiple treatment groups with unequal censoring patterns, development of a method for comparing different staging systems, a new test for exponentiality, development of survival models incorporating covariate information based on the Weibull distribution, the study of methods for choosing the best of several complex survival models for fitting the observed data, an investigation of the effects of stratification in randomized clinical trials, a study of how thresholds should be determined for diagnostic tests, and work was continued on the problem of determining the optimal treatment for patients based on initial covariate information.

PHS-6040
(Rev. 10-76)

Project Description

Many statistical problems arise in the consultative activities of the Section (see Project No. Z01-CP-04260-17-B). The investigation of these problems, the development of new statistical methods, and adaptation of existing methods for special problems constitute an important aspect of the work of the Clinical and Diagnostic Trials Section. The basic objective of this work is to enrich the repertoire of statistical methods appropriate for the analysis of clinical trial data and therefore emphasis is placed on statistical applications. However, statistical theory and application are so closely related that some of the work has a distinctly theoretical flavor. The research conducted under this project covers a wide spectrum of topics. Some of the principal subjects which have been studied during the past year are summarized below.

Treatment of Choice Problem for Clinical Trials:

Dr. Byar and Mr. Corle have continued work on developing methods for finding an optimal treatment for subsets of patients in a randomized clinical trial, even when no treatment preferences exist overall. Such methods are based on an analysis of patient covariates.

Weibull Models Incorporating Covariate Information:

Drs. Byar and Green have extended previous work on exponential survival models incorporating covariate information to the Weibull distribution. This model makes it possible to fit data where the risk of death changes in time as, for example, happens in studies of lung cancer and thyroid cancer. It was found that for patients with thyroid cancer the death rate as a function of time was less than would be predicted by the exponential assumption while for lung cancer the death rate over time was greater than that predicted by the exponential assumption.

Effect of Stratified Randomization on Size and Power in Clinical Trials:

Drs. Green and Byar have worked on an analytic formulation of the effect of stratified randomization, as compared to unstratified randomization, on Type I and Type II errors in clinical trials. Numerical results for Type I error were compared to simulation results in the literature, and new power computations were performed. The results indicate the importance in this setting of properly considering the decrease in variance that results from stratification.

Staging of Chronic Disease:

Dr. Muenz has proposed statistical tests useful in comparing alternative staging systems for chronic disease. The method was applied to comparing the TNM and End Results systems for staging breast cancer.

Selecting Survival Models Incorporating Covariate Information:

Drs. Byar, Koziol and Green have continued work on statistical methods appropriate for choosing which of several mathematical models best describes a given set of data. The models under study relate survival to a set of initial characteristics (covariates). The complexity of these models requires extensive computer studies to examine the properties of the proposed statistical tests. During the past year these methods have been used in studying data from patients with lung and thyroid cancer.

Cramer-von Mises Statistic for Randomly Censored Data:

In previous work, Drs. Koziol and Green have adapted this goodness-of-fit test to situations in which the data are subject to censorship. Dr. Koziol has now extended the methodology to encompass situations wherein the goal is to compare cause-specific survival experiences of two populations.

On Combining Independent Non-Central Chi-Square Statistics, and On Combining Independent Non-Central F Statistics:

Dr. Koziol in collaboration with Dr. Michael Perlman of the University of Chicago has studied methods for combining evidence from several independent experiments.

Exact Slopes of Certain Multivariate Tests of Hypothesis:

Dr. Koziol has developed a simple means of comparing the efficiencies of various multivariate testing procedures. He offers guidelines concerning choice of statistical methods for analyzing multivariate data sets.

On the Asymptotic Equivalence of Two Ranking Methods for K Sample Linear Rank Statistics:

Drs. Koziol and Reid derive a simple method for simultaneously analyzing data from many samples. Their method leads to simplified procedures for analyzing large sets of clinical trials data.

On Multiple Comparisons Among K Samples Subject to Unequal Patterns of Censorship:

Drs. Koziol and Reid point out that peculiarities can arise with existing methodology in the analysis of censored survival data, and suggest an improved technique.

A Simple Nonparametric Test for Trend: An Example Based on a Biological Model for Cancer of the Pancreas:

Drs. Koziol, Reid and Levitt suggest a new statistical technique for the analyses of a recent series of NCI experiments concerned with the morphogenesis of induced pancreatic neoplasms.

On Multiple Comparisons Among K Samples Differing by Scale Parameters:

Drs. Koziol and Reid investigate the Mantel-Haenszel K sample version of the statistic for comparing survival distributions, and formalize a new method of comparing these distributions on an individual basis.

A Test for Bivariate Symmetry Based on the Empirical Distribution Function:

Dr. Koziol has devised a nonparametric technique useful for examining the symmetry properties of a bivariate population.

Threshold Values for Diagnostic Tests:

Drs. Gail and Green developed a method for analyzing diagnostic test data for which the threshold value is chosen, after the results are in hand, to minimize misclassifications. This work also yielded critical values for the one-sided two-sample Kolmogorov-Smirnov test.

Testing Exponentiality:

Dr. Gail developed a convenient scale-free test for the exponential distribution. This test may be useful in clinical trials applications in which one contemplates using exponential covariate models.

Significance to the Program of the Institute:

Much of the work described above is directly applicable to the analysis of data collected in clinical trials of the treatment of cancer, analysis of data related to diagnosis and screening of cancer, and to epidemiologic studies of cancer. Beyond that the opportunity to engage in research in statistical methods is a necessary and important aspect of the work of a consulting statistician. The prestige of the National Cancer Institute as a leader in biomedical research is enhanced by having on its staff statisticians of a high caliber who have made original contributions to their own discipline. Looking at it from another point of view, the opportunity for professional recognition by means of original research publications is essential in attracting to the Institute talented statisticians of the very highest caliber. Statistical theory and applied methodology is a rapidly expanding field. If the very best and most useful methods are to be used in analyzing data related to cancer then such individuals are essential to the program of the Institute.

Proposed Course of the Project:

Many of the separate projects described in this report have been completed, but some will be continued into the next year. Noteworthy among these is the problem of selecting survival models incorporating covariate information. Drs. Byar, Koziol and Green will continue to work on this problem. Other problems which will be studied in the coming year include comparing various adjustment procedures in analyzing data from randomized trials, and developing methods for deciding how to choose the important variables for adjustment from a larger set such as that typically recorded in treatment trials. In general the statistical research projects are suggested by problems which arise in our consulting work, so we anticipate that many new problems will be studied as they come to our attention in the next year. The Section will continue a program of balanced activities divided between research and consultation, theory and application.

Publications

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CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

ADP Management Section

GEOMET, Incorporated (NO1 CP 53522)

Title: SEER and Third National Cancer Survey Data Processing Support.

Contractor's Project Director: Leon S. Pocinki, Sc.D.

Project Officer (NCI): James E. Larson

Objectives: This contract provides computer systems analysis and computer programming support to the Field Studies and Statistics Program. The contractor is developing a library of statistical and data management computer programs for use in the collection and analysis of cancer incidence and end results data.

Major Findings: The contractor is providing continuing data processing support to Field Studies investigators in the Biometry Branch and the Environmental Epidemiology Branch. Computer systems and programs and related products, and tabulations are developed by the contractor in response to requests from FS&S investigators.

Extensive support was provided to Demography Section investigators involved in the analysis of Third National Cancer Survey and SEER Program incidence data. GEOMET developed computer programs to edit and tabulate SEER data submitted this year by the Program's participating population-based tumor registries. A separate computer system was developed to assist one registry with the preparation of its data.

Approximately three man-years of effort were devoted to studies being conducted by the Environmental Studies Section.

Significance to Biomedical Research and the Program of the Institute: See Project Reports, NCI, Field Studies and Statistics Program, Biometry Branch: End Results Section, Field Liaison Section, Demography Section; and NCI, Field Studies and Statistics Program, Environmental Epidemiology Branch: Environmental Studies Section.

Proposed Course: This contract provides ongoing computer-related support and consultation services to the scientific programs of the Field Studies and Statistics Program. The need for these services is expected to continue indefinitely, although the specific programs requiring various levels of support will change over time.

The present contract expires on April 30, 1978. A two year renewal at a slightly increased level is proposed. The level of support to the Demography and End Results Sections will continue at approximately the present level. The level of effort provided to the Environmental Studies Section will increase somewhat during this period.

Date Contract Initiated: April 10, 1975.

Current Contract Level: $503,000.

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

Office of the Chief

AICHI CANCER CENTER RESEARCH INSTITUTE (NO1-CP-23213)
KUAKINI MEDICAL CENTER (NO1-CP-61060)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Professor Mitsuo Segi (NO1-CP-23213)
Dr. Grant N. Stemmermann (NO1-CP-61060)

Project Officers (NCI): Dr. James Murray and Dr. David Levin

Objectives: The purpose of these contracts is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The objective is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques have been employed, including case-control and cohort studies. The matched case-control technique is particularly useful in this kind of study. Pathology protocols have been completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis has been placed on the correlation of the epidemiological and pathology findings. In preparation for later case-control studies of prostate cancer, latent prostate cancer is being studied in 5 groups of patients. A cohort of Hawaiian Japanese males assembled by the National Heart, Lung and Blood Institute for studies of cardiovascular disease have been examined for information relevant to gastrointestinal cancers and their subsequent cancer morbidity experience will be monitored.

Major Findings: The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach while little change has been observed for breast among post-menopausal women. The case-control studies show an excess consumption of salted and dried fish and pickled "salted" vegetables some of which are rich in secondary amines, by stomach cancer patients. Since nitrites and secondary amines can be synthesized *in vivo* into nitrosamines, potent carcinogens, a possible etiology is suggested for further study.

A study of indigenous Japanese from Akita Prefecture, Japan, and Hawaii Japanese in Honolulu indicated that 24.7% of patients with intestinal

metaplasia in at least 2 or 3 pre-selected sites within the stomach had a low serum group I pepsinogen level. The specificity of a low level for the presence of severe metaplasia was 97.7%. In two groups of male, age-matched subjects--Hawaii Japanese and Hawaii Caucasians--the frequency of a low serum group I pepsinogen level was four-fold greater in the Japanese than in the Caucasians.

Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. A possible resolution of this anomaly has been contributed by Dr. Akazaki, who has described and defined two types of latent carcinoma--proliferative and non-proliferative. The gradient in prevalence of the proliferative type lesion in his autopsy studies of specimens from Japan and Hawaii Japanese corresponds closely to the gradient in incidence of clinical prostatic carcinoma in the two populations. The important feature of the Hawaii Japanese males is the greater amount of proliferation and not their unremarkable overall prevalence of occult lesions, which suggests the presence of some promoting factor in the migrant male population.

Case-control studies of Hawaiian Japanese revealed excess risks of bowel cancer for persons who regularly ate Western-style meals only. The similarity in findings for Issei and Nisei sharply contracts with results previously reported for stomach cancer in Issei and Nisei, where the reduced risk among persons regularly eating Western-style meals only was limited to the second-generation Nisei. Bowel cancer was associated with consumption of specific foods. Bowel cancer patients ate meats, legumes, and starches more frequently, with the case-control disparity being less pronounced for individuals consuming starches than for those eating meat and legumes. Beef and string beans were the major contributors to the meat and legume effects. There seemed to be separate effects for beef, string beans, and starches, and the case-control contrasts were strengthened when various combinations of foods were considered. The findings have stimulated the search for etiologic factors and the studies in Hawaii and Japan have been used in the search for differences in intestinal flora and fecal chemistry between the native and migrant Japanese. Specimens for bacteriological and chemical study have been and are continuing to be made available to other investigators supported by NCI research contracts and grants.

Significance to NCI Program and Biomedical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicate those that are predominately environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course: These two contracts are expected to continue for several years because of the long range nature of these prospective studies.

Date Contract Initiated: February 1972 (NO1-CP-23213)
June 1971 (NO1-CP-61060)

Current Annual Level: $153,798 (NO1-CP-23213)/$410,500 (NO1-CP-61060)

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
FISCAL YEAR 1977

Office of the Chief

CALIFORNIA STATE DEPARTMENT OF HEALTH (NO1-CP-33353)
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NO1-CP-43383)
CHARITY HOSPITAL OF LOUISIANA (NO1-CP-43262)
CONNECTICUT STATE DEPARTMENT OF HEALTH (NO1-CP-43203)
FRED HUTCHINSON CANCER RESEARCH CENTER, SEATTLE (NO1-CP-33246)
RESEARCH CORP. OF THE UNIVERSITY OF HAWAII (NO1-CP-53511)
UNIVERSITY OF IOWA (NO1-CP-43200)
MICHIGAN CANCER FOUNDATION (NO1-CP-43264)
UNIVERSITY OF NEW MEXICO (NO1-CP-33344)
UNIVERSITY OF UTAH (NO1-CP-43382)
YALE UNIVERSITY (NO1-CP-33235)
EMORY UNIVERSITY (NO1-CP-61027)
GEOMET, INC. (NO1-CP-53522)
COMMONWEALTH OF PUERTO RICO (NO1-CP-43386)

Title: Surveillance, Epidemiology and End Results Reporting (SEER) Program

Project Officer (NCI): Max H. Myers, Ph.D.

Objectives: To obtain and analyze data on cancer morbidity, mortality and patient survival from population-based registries, to identify areas for epidemiologic investigations, and to initiate preliminary investigations needed to develop epidemiologic study protocols.

Methods Employed: The National Cancer Institute is sponsoring a collaborative program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER) Program. Participants in this program are all population-based registries, covering entire states or geographically specified areas, usually Standard Metropolitan Statistical Areas. The Biometry Branch has professional and technical responsibility for supervising these contracts. Basic information is collected on all patients treated for cancer in the area, area residents treated outside the area, as well as anyone dying in the area where cancer is mentioned anywhere on the death certificate--or area residents dying outside the area, where cancer is mentioned anywhere on the death certificate. Periodic follow-up information is obtained on the vital status of all those in the registries. Data are submitted annually to NCI on magnetic tape according to a specified format. These data are available for analysis as well as for providing an extensive resource for special studies.

Core epidemiological staff are present in some of the SEER Programs. These staff members will review the morbidity data to identify epidemiological research leads that should be investigated. This staff will develop study protocols and field test the study schedules. Funding for such proposals would be either through other contract or grant support or financial assistance from other sources.

Significance to NCI Program and Biomedical Research: Information on incidence, survival and mortality from a representative cross section of the U.S. population is required on a continuing basis so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variation in cancer incidence and survival with respect to geographic and demographic characteristics of the population and differential changes over time, specific etiologic hypotheses will emerge which, when tested via special study mechanism, should lead to the identification of controllable risk factors. Such research is an integral part of the ultimate goal of the national program: the reduction in the occurrence of and mortality due to cancer. Continued recording of cases will also provide a data base that could be useful in the evaluation of cancer control activities. These data could provide the baseline against which changes resulting from specified cancer control programs could be measured to determine the effectiveness of these programs in reaching their stated goals. A system of record linkage between the cancer registry and employees of specific industries or specific types of occupations would provide early warning of possible occupational carcinogenicity. This would significantly reduce the time when cancer hazards would be identified.

Major Findings:

Data for cases diagnosed between January 1, 1973 and December 31, 1975 were submitted to NCI in January, 1977. Two participants did not submit data for 1973. While it is felt that certain areas do not have complete reporting for all years, the data were deemed to be sufficient to calculate incidence rates to permit geographic comparisons by primary cancer sites.

Currently, data are available on 191,437 cases among residents of the nine SEER areas who submitted data. Of these 173,201 cases were malignant lesions, 14,643 were in situ lesions and 3,593 cases were reports of benign tumors which were specifically reportable to the program. Preliminary analyses of these data have centered around the malignant lesions.

Preliminary estimates of the incidence of invasive cancer excluding nonmelanoma skin cancer for all SEER areas combined for 1973, 1974 and 1975 respectively are 313.3, 325.2 and 313.0 per 100,000 population adjusted to the 1970 population of the United States. This compares to a rate of 300.0 per 100,000 for the nine areas reporting to the Third National Cancer Survey 1969-1971. The increase in 1974 was due primarily to an increase in breast cancer reported among white females during that year: 89.8 per 100,000 females compared to 78.2 in 1973 and 82.5 in 1975. Also, in 1975 there was a decline of about 4% in the incidence rates for males. This decline is primarily due to the absence of data from Veterans Administration Hospitals which due to legal restrictions did not report data to the SEER Program.

The rates cited above were calculated based on staff projections of populations of the various SEER areas. Currently, the Census Bureau through an interagency agreement is developing official population estimates by sex and age for whites and nonwhites for each county in the United States. When these estimates become available the preliminary rates are expected to change. These rates will also be expected to change both when data become available from VA Hospitals and as two areas correct the underreporting of their data.

Hence, the 1973-1974-1975 data served as an additional basis for quality control and field audit investigations. By comparing the trend in data over the three year period, as well as among the programs, items for investigation of possible errors of underreporting were identified.

Two program participants and NCI staff have continued collaboration on the development of a sophisticated data management system for collecting and maintaining patient and disease oriented information on a large scale. Information, detailed plans and computer programs will be shared between these groups. System components with documentation will ultimately be available to other SEER participants and to other NCI programs with similar data handling requirements. Progress this year includes the completion of comprehensive system design documentation and computer programs for a core system which will be operational in one SEER Registry late this year.

To assist in the training program and to provide guidance to SEER Program participants and others concerned with cancer registration, a number of publications have been developed and distributed. These include manuals that set out clear guidelines for collecting and coding the data to be submitted to NCI, as well as booklets that can be used for on-the-job training of personnel responsible for carrying out the functions required for data extraction and coding. The demand for these publications has been large and mailings have been made to a variety of medical personnel and facilities.

Four participants in the SEER Program who had formerly been a part of the End Results Program submitted data on cancer patient survival to the NCI in September 1975. According to these data, there have been some improvements in the relative survival rates for all forms of cancers combined since the 1960's. 1/

	Year of Diagnosis	
	1960-66	1967-73
White		
1-year	61	64
3-year	45	47
5-year	40	41*
Black		
1-year	50	54
3-year	33	37
5-year	28	32*

*Complete 5-year follow-up not available for cases diagnosed 1970 or later.

1/ The relative survival rate is defined as the ratio of the observed survival rate for the patient group to the rate expected of persons in the general population similar to the patients with respect to age, sex, race and calendar year of observation.

While these improvements are not large, they are nevertheless improvements. However, there is potential fallacy in examining survival rates for all forms of cancer combined. If there had been an increase over time in the relative frequency of forms for which survival was poor, a no-increase trend for all forms combined would suggest that there are some types of cancer for which survival has improved. This has in fact happened since there has been an increase in the relative frequency of lung cancer from 1960-66 to 1967-73 from 11 to 13 percent in white patients and 12 to 15 percent in black patients.

Additional details on findings may be found in progress reports for Project Numbers Z01-CP-04254-03-B and Z01-CP-04257-20-B.

Proposed Course of the Project:

During the development phase of the SEER Program activities, intensive effort will be expended to assure that the data being submitted to NCI are of the quality and completeness required for an effective cancer surveillance networt. Once this level of reporting is reached, the quality control activity will be geared toward the needs of the individual programs and will depend on each one's level of data quality. The Field Liaison Section staff in cooperation with the Training staff of the University of California Medical School in San Francisco will conduct this program of quality control visits, as well as national and regional workshops, intensive formal training programs and will continue to develop various kinds of instructional materials.

A study of the cost associated with abstracting and coding the detailed extent of disease and diagnostic procedures data is underway. This cost must be compared with the cost for acquiring summary extent of disease data. Results from this study will provide some guidance regarding implementation of detailed coding schemes for cancer sites other than colon, rectum and breast which are already in force. Coding instructions have been developed and field tested for lung, stomach, uterine cervix, uterine corpus and prostate. Similar descriptive schemes will soon be available for melanoma of the skin, bladder and the lymphomas.

	Year Started	FY-76 Negotiated
California State Department of Health (NO1-CP-33353)	1962	$1,052,556
University of California, San Francisco (NO1-CP-43383)	1974	55,000*
Charity Hospital of Louisiana (NO1-CP-43262)	1973	178,709
Connecticut State Department of Health (NO1-CP-43203)	1963	402,383*
Fred Hutchinson Cancer Research Center (NO1-CP-33246)	1973	220,000*
Research Corp. of the University of Hawaii (NO1-CP-53511)	1971	526,761
University of Iowa (NO1-CP-43200)	1973	448,585
Michigan Cancer Foundation (NO1-CP-43264)	1973	814,068
University of New Mexico (NO1-CP-33344)	1973	302,360*
University of Utah (NO1-CP-43382)	1973	350,000*
Yale University (NO1-CP-33235)	1973	499,302
Emory University (NO1-CP-61027)	1976	321,522
Geomet, Inc. (NO1-CP-53522)	1975	503,000*
Commonwealth of Puerto Rico (NO1-CP-43386)	1968	40,000*

*Estimate

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

Office of the Chief

FUNDACION PARA LA EDUCACION SUPERIOR (NO1CP33286)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Dr. Carlos Cuello

Project Officer (NCI): Dr. James Murray

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia, to correlate the morbidity data with pathology findings, and to conduct epidemiological studies of specific sites based on leads suggested by these data.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socioeconomic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys of a limited scale and duration are being conducted in Cartegena and Medellin, cities thought to have greatly different site-specific incidence.

In Cali an organ bank of tissues from persons dying from accidents and acute diseases is being utilized for study of occult tumors and malignant precursors with emphasis on stomach, colon and cervix. The organ bank findings also emphasize the distribution by place of birth and are used to complement the incidence data from the morbidity surveys. Similar pathologic material is being collected in new Orleans and Hawaii. Autopsy studies directed to suspect precursor lesions of cancer of the stomach (intestinal metaplasia) and of the large bowel (polyps) are being conducted in Cali, Medellin, San José (Costa Rica) and São Paulo (Brazil).

Major Findings: Cancer incidence data have been produced for the city of Cali for the 5-year period 1967-1971 and have been published in the new third edition of Cancer Incidence in Five Continents. Cancer incidence data is also being collected annually in the city of Medellin on a major portion of the working force of that population covered by the local social security system. Studies are continuing on the precise localization of large bowel cancer lesions and their histologic characteristics. Cali residents are at low risk for large bowel cancer, much of which is accounted for by a deficit of tumors in the sigmoid colon. Cali has been shown to have a strong social class gradient in colon cancer with the upper classes having risks 2.5 times those of the lower classes. Such a gradient is not seen in North America nor Western Europe. The social class differences in anatomical location of large bowel tumors mimic those found in contrasts of high-risk and low-risk countries.

In the Department of Nariño in the southern part of Colombia, the comprehensive epidemiologic study of stomach cancer has revealed strong clustering of cases that may be associated with nitrates in water supplies and selected food items. The observational situation is very favorable for pursuit of diet and stomach cancer relationships. A set of three major papers based on this research were published in November, 1976 describing in detail the cancer risk associated with suspected environmental agents, case-control studies of precursor lesions in the stomach, and the natural history of precursor lesions. We hope that this information will eventually lead to identification of the underlying causes of stomach cancer and their subsequent elimination.

A major study of cervical cancer and precancerous lesions in the Cali population is now in the analytical phase. Autopsy specimens and associated information has been collected over the past eight years, and all microscopic diagnoses have been checked for reproducibility. This research also includes a study of the prevalence of intra-epithelial neoplasia of the cervix in Cali women during the periods of 1960-1967 and 1968-1975. The aim of this study is to compare the time trends of these lesions before and after the initiation of a large cervical cytology screening program in Cali.

Significance to NCI Program and Biomedical Research: This contract is producing significant data for comparative studies in the United States and elsewhere, with emphasis on areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: The various aspects of this project will continue for several years. Emphasis will be placed on clinical and experimental studies of the genesis of intestinal metaplasia and carcinoma of the stomach. New collaborative research efforts will begin with investigators in Brazil and Costa Rica.

Date Contract Initiated: June 29, 1966

Current Annual Level: $96,566

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
FISCAL YEAR 1977

End Results Section

HEALTH INSURANCE PLAN OF GREATER NEW YORK (NO1-CP-43278)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Dr. Raymond Fink

Project Officer (NCI): Dr. Philip C. Prorok

Objectives: A. To determine the frequency with which a screening technique using mammography and clinical examination can detect early breast cancer.

B. To establish whether such screening holds substantial promise for improving survival rates among women with newly diagnosed breast cancer and for lowering breast cancer mortality in the female population.

C. To investigate, through a prospective study of women screened, the relationships of a wide range of parameters to the development of breast cancer.

D. To store plasma from a large group of women that can be retrieved for use in hormonal and immunological studies related to risk factors and etiology of breast cancer.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving clinical examination plus mammography at annual intervals and a control group of approximately 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966.

During one reexamination cycle 40 cc of blood was taken from women in the study population and stored.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes clinical examination plus mammography. Over the short run period of 7 years of follow-up, the study group of women has about one-third less mortality from breast cancer than those in the control group. This reduction in mortality appears to be concentrated among women over 50 years of age. The differential between control and study cases in fatality from breast cancer is almost entirely due to the exceptionally low rate among

the cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation, but the magnitude of the independent contribution of each modality is difficult to determine.

In examining various risk factors for the women who appeared for an initial screening, the following women were found to have a 20-30% increase in risk of breast cancer: higher educated women, Jewish women, and unmarried women. Increasing age at first pregnancy, low gravidity, and menarche at ages under 15 are associated with a 50-100% increase in risk.

Significance to NCI Program and Biomedical Research: Early diagnostic procedures offer considerable promise for the secondary prevention and potential cure of cancer. It appears that the combination of clinical examination and mammography is such a procedure and is being extended to other populations. The role of mammography alone in this process is being investigated.

Proposed Course of Projects: The screening part of the study is complete and the emphasis from now on will be on follow-up and data analysis. The women will be followed for as long a time period as is necessary to demonstrate whether the technique is truly useful as an early diagnostic device having a favorable long term impact on mortality from breast cancer. The duration of this study is contingent upon the answers to the questions implied by the objectives.

Supplemental lines of inquiry may be suggested by the Breast Cancer Task Force. Proposals for use of the stored blood plasma are being developed.

Date Current Contract Initiated: November 1, 1962

Current Annual Level: $174,720

CONTRACT NARRATIVE
BIOMETRY BRANCH, DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

Clinical and Diagnostic Trials Section

INFORMATION MANAGEMENT SERVICES, INC (NO1CP71010)

Title: Computer Services

Contractor's Project Director: Janis Beach

Project Officer (NCI): Donald K. Corle

Objectives: This program provides systems design and computer programming support for projects in the Clinical and Diagnostic Trials Section.

Major Findings: The contract was initiated on April 11, 1977 and replaces contract NO1CP53524 previously held by Mason Research Institute. IMS has performed one update of the prostate and bladder cancer data systems. IMS personnel worked independently using documentation prepared by the previous contractor. Special effort was made in securing follow-up information on patients in one bladder cancer trial.

IMS personnel have written programs to aid in the analysis of data from a study of sickle-cell anemia. Programs were also written to help in the preparation of a final report for a study of thyroid cancer conducted by the EORTC data center in Brussels, the determination of the importance of tumor histology in predicting deaths from prostate cancer, and the analysis of disease progression in Stage I and II prostate cancer patients treated with prostatectomy.

Proposed Course: Although IMS personnel have worked with the Section for only a portion of Fiscal Year 1977, the contractor has been very responsive to the needs of the Section. The contractor's major responsibility will be in the development of editing, file maintenance and statistical programs for the research projects conducted by the Section.

Date Contract Initiated: April 11, 1977

Current Contract Level: $102,563

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
FISCAL YEAR 1977

End Results Section

ISRAEL CENTER FOR REGISTRATION OF CANCER AND ALLIED DISEASES (NO1-CP-33351)

Title: Continued Cancer Registration and Selected Studies

Contractor's Project Director: Dr. Leah Katz

Project Officer: Mr. William I. Lourie Jr.

Objectives: Building upon the effective reorganization of the Israel Cancer Registry accomplished by previous contractual agreements, to continue the country-wide registration of cancer data and its utilization in a variety of statistical epidemiologic studies, to initiate selected studies requiring the acquisition of additional information from other record sources.

Major Findings: Increased general usage of the "Identity Number" of the Israel Population Register has improved identification of duplicate reports. Continuing liaison with the Population Register provides checks upon birth and death dates as well as cohort estimates for year of immigration. Similar linkage with files of the Central Statistical Bureau validates data on cause of death. Such procedures help assure quality control. The files now contain approximately 120,000 registered cases. In addition to an analysis of the 12 years 1961-1972, the data have been recast to the format of certain tables for site and histology in NCI Monograph 41 concerning the Third National Cancer Survey. Also, data for 1967-1971 were submitted and published in Volume III of "Cancer in Five Continents" in the format and detail requested by IARC for five population groupings by sex, age and primary site. The contractor field-tested the new ICD-O code for WHO. Studies have been initiated concerning the joint occurrence of cancer and tuberculosis, the possible affect upon childhood leukemia incidence by BCG immunization at birth, the identification of cancer "clusters" in families. Dr. Leah Katz has become the Director of the Registry after retirement of Dr. Ruth Steinitz who is, however, still active in the analysis and planning of research studies. An advisory board has been established which will help determine research priorities and assure the coordination of any collaboration with clinicians and hospitals in specific field studies.

Significance to NCI Program and Biomedical Research: Because of the large number of ethnic and immigrant groups, Israel's cancer data have unique epidemiologic potential and fit well into the Branch's collaborative program concerning studies of change in cancer incidence and mortality after migration. Of major interest is the fact that specific differences in cancer incidence between population subgroups are reported from Israel which far exceed those found between different population groups in the U.S. Data from the population-based Israel Cancer Registry can also be usefully integrated in analyses of data generated by the Branch's SEER Program which is also population-based.

Proposed Course: Continued support of the registry, especially for initiation and conduct of special studies of interest to the SEER Program. (For example, among those proposed are the contractor's first survival analyses, including a pilot study for colon and rectum by stage and therapy.) The result is a slight increase in the need for time of professional personnel. Plans are for a formal site-visit in November 1977.

Date Contract Initiated: June 28, 1972

Current Contract Level: $86,000 (April 1977 - March 1978)

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

Office of the Chief

LOUISIANA STATE UNIVERSITY MEDICAL CENTER (NO1CP53521)

Title: Studies of Epidemiology and Geographic Pathology of Cancer

Contractor's Project Director: Dr. Pelayo Correa

Project Officer (NCI): Dr. James L. Murray

Objectives: This project has been expanded since last year and now involves several objectives. The search for premalignant lesions in the large intestine is continuing, and attempts are being made to correlate the frequency of adenomatous polyps with other factors such as diet, cholesterol metabolism and atherosclerosis. Laboratory experiments will be conducted on the possible inhibition of nitrosamine synthesis in order to test part of the hypothesis on gastric carcinogenesis. Collaborative international studies will be continued on prostatic cancer, breast cancer and premalignant cervical lesions. A broad objective of this project is to establish collaborative research programs on epidemiology and geographic pathology of cancer in various Latin American countries.

Methods Employed: Premalignant lesions in the large intestine are identified by a careful gross and microscopic study of bowel specimens obtained at autopsy in New Orleans. In the studies on inhibition of nitrosamine synthesis, the Ames test which is based on the mutagenic effects of carcinogens in bacteria will be used as well as various methods to inhibit the induction of cancers in rats by known carcinogens. In order to develop new programs of research in cancer epidemiology and geographic pathology in Latin America, special centers or groups of university-affiliated researchers with competence, capability and interest are being identified and contacted. An important first step in the development of such programs is the establishment of a good local cancer registry.

Major Findings: There is a good correlation between the prevalence of adenomatous polyps and colon cancer risks in various communities. During the past three decades the polyp prevalence in whites has doubled and has increased more than four times in blacks. This study of autopsy specimens also indicates that there is no longer a higher prevalence in males. Recent work in collaboration with investigators in Cali, Colombia, has concentrated on premalignant lesions of the stomach and has resulted in a proposed etiologic hypothesis which calls for intragastric synthesis of carcinogenic nitrosamines from nitrates and amines in the diet. Three papers have been published on the stomach cancer research in Colombia describing the results of a case-control study, a population survey of residence, occupation and dietary history, and the natural history of the development of precursor lesions.

Other work on colon cancer is also focusing on effects of diet and intestinal flora. Collaborative research with Dr. Akazaki in Japan has shown that the prevalence of latent (occult) prostate carcinoma does not agree with the incidence and mortality data in different countries. The findings indicate the more important role or promoter factors than initiators.

Significance to NCI Program and Biomedical Research: The recent development of working hypothesis on the etiology of stomach cancer is considered to be a major step forward in our understanding of the causes of this disease. Definitive studies are now being carried forward to test this hypothesis. It is also hoped that this research project will identify those epithelial lesions which are epidemiologically related to colon cancer. This will not only provide clues on the pathogenesis but will identify individuals at high risk for colon cancer and provide a signal of exposure closer in time to the contact of patient and environmental factors. The cooperative studies in Latin American countries will offer unique opportunities to identify important factors associated with the etiology of various cancers.

Proposed Course: This contract will continue for several years and will serve as a focal point for collaborative studies in Latin America.

Date Contract Initiated: January 1, 1975

Current Annual Level: $338,199

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1977

Office of the Chief

NORWEGIAN PUBLIC HEALTH SERVICE (NO1-CP-43247)
UNIVERSITY OF MINNESOTA (NO1-CP-33325)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (NO1-CP-43247)
Dr. Leonard Schuman (NO1-CP-33325)

Project Officer (NCI): Mr. Joseph Scotto

Objectives: To determine the reasons for difference in cancer incidnece and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic and demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: The University of Minnesota has defined a cohort of approximately 20,000 policyholders in an insurance company that has a high proportion of persons of Norwegian descent and will monitor the group prospectively for mortality data. The contractor has also carried out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with the ongoing work in Norway.

The introduction of a personnel identification number system in Norway has made record linkage a simple operation, made a large number of studies possible, and facilitated keeping track of cohorts used in the migrant studies. The personnel identification number system is being used to link current study records with data from the 1960 Norwegian census to better identify the characteristics of various population groups. This enabled a study of nickel workers to be accomplished at a much lower cost than would be possible in the United States of America.

The Norwegian Registry also provides one of the means for doing ad hoc international studies. For example, there is interest in studying the effect of differences in incidence rates for such sites as breast and large bowel on the stage at diagnosis and the survival rates. The studies are done more effectively using different countries for study populations. A comprehensive report on patient survival for all cancer cases diagnosed in Norway between 1953 and 1967 is being readied for publication.

Significance to NCI Program and Biomedical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: Although plans for continued analyses of data (e.g., on respiratory cancers and gastrointestinal cancers) were made last year, little has been realized during the past report period. Principal investigators (Dr. Schuman and Dr. Bjelke) have requested that a supplement to the contract be made to support a full-time, experienced epidemiologist to help with data analyses and publication of results. Over eight-years of follow-up have already been realized, and it is important to begin now the serious business of in-depth epidemiologic analysis with these data. Should the data be found wanting in certain informational items (such as histology codes or demographic factors) they may be more easily collected while the study is still in progress. By the time a full 10 years worth of follow-up will have accumulated all data problems will have been resolved, thus providing a more complete and comprehensive final analysis. With additional professional support, new studies may also be started on breast cancer, thyroid cancer and Hodgkin's disease.

Date Contract Initiated: March 1964 (NO1-CP-43247)
June 1966 (NO1-CP-33325)

Current Annual Level: $105,345 (NO1-CP-43247)
$ 22,557 (NO1-CP-33325)

SUMMARY REPORT
CLINICAL EPIDEMIOLOGY BRANCH
JULY 1, 1976 THROUGH SEPTEMBER 30, 1977

The primary purpose of the Clinical Epidemiology Branch is to determine who is especially susceptible or resistant to cancer, and why. Specifically, studies are made of peculiarities in the occurrence of cancer in persons, families, or ethnic groups. In certain circumstances batteries of laboratory tests are applied to elucidate subclinical abnormalities that may portend a high-risk of cancer or reveal new understanding of its pathobiology. Hypotheses generated in the experimental laboratory are tested for their applicability to man. Conversely, the patterns of disease occurrence in man may suggest biological concepts which can be elucidated in the experimental laboratory. Evidence is sought for the interaction between host factors and environmental agents. Steps are taken to develop or improve resources for clinical etiologic research. We encourage the reporting of etiologic observations as derived at the bedside by alert clinicians. Workshops are convened on promising but unfashionable subjects, and the Branch serves as a communication center for recent clinical advances in the study of cancer causation.

Staff Changes:

There were two additions to the Branch during the year: Judith L. Bader, M.D., a pediatric oncologist became a Clinical Investigator in October 1976, and Gilbert W. Beebe, Ph.D., a biostatistician, became a consultant for two years beginning in May 1977.

Research Program:

In December 1975 the Epidemiology Branch was divided into separate Environmental and Clinical Branches. In the 1-1/2 years since, the Clinical Epidemiology Branch (CEB) has essentially had to build a program from scratch.

Activities within the United States:

Studies of patients within NIH: A program has been initiated of obtaining special etiological histories from patients of interest on the surgical wards. (The surgeons proved to be more interested and to have a better array of patients than did the pediatricians or internists.) This activity was coupled with a review of all 287 surgical hospital charts since the service originated in 1954 through December 1974. Both endeavors yielded cases and series of etiologic interest concerning cancers of soft tissues or bone. For example, among the new cases, the most dramatic was a deaf black male with blue eyes, premature grey hair and osteosarcoma. The findings resembled those of a genetically transmitted disorder, Waardenburg's syndrome, which is not known to predispose to osteosarcoma. Careful examination revealed that the patient also had an unusual facies, thumbs and toes misplaced downwards, hypogonadism, and albinoid fundi. In the usual clinical examination, he would simply have been diagnosed as a deaf man with osteosarcoma. The array of observations

may afford an opportunity to describe a disease not previously identified, and to search by laboratory studies for abnormalities that may account for the occurrence of bone sarcoma. In any event, detailed study of this patient established the value of the etiological approach on the surgical service, where our staff now enjoys special referrals.

To date we have interviewed 65 current sarcoma patients. The information obtained has been added to the medical charts as etiological consultative notes. The patients seem willing to help us seek clues to the causes of their illnesses. In addition, our staff often responds to the patient's concern about the possible roles of heredity, diet or trauma in the genesis of their tumors.

Review of the surgical case-series has drawn attention to several past patients of special interest; e.g., a woman with fibrosarcoma of the face several decades after radiotherapy for acne, who may have been especially predisposed to neoplasia, since both she and her sister have developed breast cancer. Two patients were noted with osteosarcoma about 20 years after the diagnostic use of thorotrast, a radioactive compound formerly used in diagnostic radiology. Thorotrast is well known as a cause of leukemia, but osteosarcoma has rarely been reported, perhaps because children (as in these cases) were rarely given the compound. Despite these individual findings, the case-series revealed that soft-tissue tumors (i.e., of muscle, fat or connective tissue primarily) tend to be associated with unusual findings in the patient or family more often than is found for bone tumors. Thus there is an unequal distribution of host and environmental risk factors.

The experience on the surgical service of NCI has led to a similar approach in National Heart, Lung and Blood Institute. There a substantial series of tumors that raise blood pressure have been under study. The tumors are pheochromocytomas, chemodectomas, and carcinoids. The Clinical Director of the Institute has kindly invited our staff to abstract past records and to interview present patients. Interest focuses particularly on the family histories for evidence of genetic disorders that predispose to these tumors, knowledge of which is important in early detection, and in genetic counselling of persons in the child-bearing age.

In this endeavor, we have experimentally employed professional personnel who are not physicians to obtain etiologic histories, and they have displayed great interest and aptitude in their work.

Studies of patients outside NIH: To extend our concept beyond NIH, we have long had a small unit at the Sidney Farber Cancer Center in Boston. There the studies of cases and case-series has led to a substantial series of publications. Among those that have evoked the most interest are reports concerning the health and reproductive capacity of children who survive cancer, and the occurrence of second primary tumors, either inherent or due to therapy for the first tumor.

A pediatric oncologist, new to our staff, has been establishing a working relationship with the cancer unit of the National Children's Hospital in

Washington, where we hope to encourage etiologic studies and to acquire laboratory specimens, which may reveal something new about the genesis of specific cancers. This research could lead to tests for screening environmental agents for carcinogenicity or to predict who is at high risk, so important in prevention and early detection.

It is understandably more difficult to stimulate clinical etiology by making ward rounds, by lecturing and holding seminars at intervals at hospitals outside Washington. The Children's Hospital in Cincinnati has, however, established a weekly meeting of its oncology unit, specifically for this purpose. Other hospitals at which our staff lectured or made rounds during the year include the University of Virginia, St. Jude Hospital in Memphis, University of Maryland, Walter Reed Army Medical Center, M.D. Anderson Hospital, Johns Hopkins Hospital, Long Island Jewish Hospital, NCI, George Washington University, Howard University, and Columbia University.

Family studies: In the study of cancer families there is considerable overlap with the Environmental Epidemiology Branch. The families of interest are referred to us by NCI, and by physicians in various parts of the country. The unusual aggregation of cancer in a family affords an opportunity to use newly developed laboratory procedures in an attempt to define underlying abnormalities which may clarify the fundamental biology of cancer. The cancers in family members are at times of the same type, but at other times are of dissimilar type, or are associated with certain non-cancerous diseases -- as if different reactions to the same stimulus were occurring. About 20 new families have been ascertained for study since October 1976. Among the most dramatic developments of the year was the occurrence of acute myelogenous leukemia (AML) in a young woman whose 3 sibs and 3 cousins had died of the disease. In 1969 she was thought to be at high risk because her skin fibroblasts underwent excessive malignant transformation in culture when infected with the Simian Virus 40 (SV40), which causes cancer in newborn experimental animals. Her brother who had AML and her mother also showed increased transformation. The laboratory test seemed to predict the development of AML in this particular instance. When the diagnosis was made the mother said, "I thought so, because she now has the same smell that the other three had." We now plan to investigate the origin of the odor, and the possibility that some cancers may be detectable by people, (animals?) or machines that can detect subtle odors. The patient's fibroblasts will also be studied for defective DNA repair.

Among other families under study is one in which 4 of 13 sibs had cancer (2 Hodgkin's disease, 1 nasopharyngeal carcinoma, and 1 embryonal carcinoma of the testis) and 3 were mentally retarded, possibly as part of an ill-defined syndrome. Various members of the family have immunological abnormalities involving lymphocytes.

In another family the index case has had 14 primary cancers in 5 organ systems (colon, skin, stomach, breast, and endometrium), and all 3 of her sibs and both parents have had cancers.

The charts for all patients seen by Dr. Helen Taussig of Johns Hopkins Hospital, 1945-51 (N=about 1200) for congenital heart disease are being reviewed for the occurrence of cancer. Follow-up covers an interval of 28 years. Preliminary results suggest that a specific tumor is occurring more often than expected in the group.

Laboratory studies: A wide array of laboratory tests are applied to individual patients, case-series, and families with peculiar occurrence of cancer, sometimes in conjunction with other disorders. Of great interest at present is the inability of persons with certain inherited diseases to repair damage to DNA produced by radiant energy (x-ray or ultraviolet). These people have a high frequency of certain cancers, which may (or may not) be related to the DNA repair defect. Tests of DNA repair, and a more sensitive measure of genetic damage of unknown significance (increased sister-chromatid exchanges) are being applied to the members of the AML family mentioned above, among others. Also an attempt will be made to study persons or domestic animals with unexpected severe acute reactions to conventional doses of radiotherapy or sunlight.

Demographic studies: Our computer technologist has developed a series of programs for graphic depiction of cancer mortality by counties in the United States, 1950-1975. Our Consultant in Biostatistics, who recently joined the Branch, plans to evaluate this material for a publication on peculiarities in cancer mortality with respect to demographic variables.

The frequency of cancer mortality in men who served as radiology technicians vs. medical/pharmacy technicians in World War II was evaluated previously in an 18-year follow-up study (through 1963). The follow-up study, extended to 29 years -- through 1974, revealed no statistically significant excess in mortality from specific cancers among the radiology technicians, whose total mortality had risen from 55 to 792 deaths from all causes.

An evaluation of data on teratomas in man and domestic animals shows unusual occurrences which must signal as yet unknown biological processes in the genesis of these tumors with respect to anatomic location, species, and strain (e.g., low frequency of testicular teratomas in Blacks, or the high frequency of pineal teratomas in Japanese).

By contacting several major pediatric cancer units in various parts of the country and by reviewing data on file in the Branch, we almost doubled the number of previously reported cases of leukemia in persons with multiple neurofibromatosis, an autosomal dominant disorder. The leukemia differs from usual in being predominantly of the non-lymphocytic type; i.e., chronic myelogenous (juvenile type) and acute monomyelogenous leukemia. The association between multiple neurofibromatosis and leukemia defies the generalization that persons at high risk of leukemia have chromosomal abnormality as a feature in common.

Other activities: Three members of the Branch have contributed substantially to the activities of the Committee on Environmental Hazards of the American Academy of Pediatrics, especially as they relate to the special

susceptibility of the fetus and child to environmental agents including carcinogens. This subject has not been of compelling interest to pediatricians, and it has been our purpose to increase their awareness of the potential hazard of such pollutants as parental cigarette-smoking, while keeping the profession informed on the current understanding of pollutants in breast-milk (e.g., insecticides, PCBs, and PBBs).

Activities in Japan:

Radiation Effects Research Foundation (RERF): Since 1971, the Branch has provided funds through a contract to RERF to establish a Visiting Scientist Program under which 2-4 young Japanese medical scientists or statisticians from universities agreed to work in Hiroshima for one year during which they spent two months in the U.S., where they attended summer sessions on epidemiology or statistics and visited several leading epidemiologic research programs. The contract also provided for the establishment in Hiroshima and Nagasaki of registries for histologic specimens from tumors obtained primarily by biopsy or at surgery. The contract is now being phased out, because its purposes have been served. The feasibility of the Visiting Scientist Program has been demonstrated, and both tissue registries are now well underway. The results to date are being tabulated, and will be presented at various Cancer Centers in the United States in September 1977 by pathologists associated with each registry.

U.S.-Japan Cooperative Cancer Research Program: A meeting was held of the Committee on Analytical Epidemiology in Tokyo on October 8-9 to discuss cancer mortality among the Japanese as compared with U.S. whites and blacks with respect to age, sex, calendar year, and anatomic site. Earlier in the year, Dr. T. Hirayama, co-chairman of the Committee had been given the U.S. data by the Epidemiology Branches of NCI. Dr. Hirayama has since published these data as a volume: Comparative Epidemiology of Cancer in the U.S. and Japan -- Mortality, issued in January 1977. The remainder of the conference was devoted to recent developments in childhood etiology as studied in Japan, and in the epidemiologic findings of 1) urinary bladder cancer as related to chemicals, 2) uterine cervical cancer as related to a transmissible agent, and 3) leukemia, as related to chromosomal aberrations.

The annual meeting of the delegations from Japan and the U.S. was held in Kyoto on October 12-13, at which time plans were furthered for a meeting of the Committee on Analytical Epidemiology to be concerned with biostatistics, for which there is great potential but little tradition as yet in Japan. This meeting would be held in the summer of 1978.

Under the Program, Minako Nagao, Ph.D., who is engaged in studies of chemical carcinogenesis in the laboratory of Dr. T. Sugimura at the National Cancer Hospital Research Institute in Tokyo, spent one week in our Branch in April 1977.

Other international activities:

Seveso, Italy: At the request of the National Academy of Sciences (NAS) Dr. Miller served with a four-member group to consult with Italian officials

and scientists in Rome and Seveso, April 2-6, 1977 concerning follow-up studies of persons exposed to dioxin when a runaway reaction at a factory spewed a chemical cloud over a neighboring community of about 5,000 persons. A report was submitted by the consultants to the Executive Committee of the NAS, suggesting possible future collaborative activities in the study of the effects of dioxin and means for decontamination.

West Berlin: Dr. Miller prepared a background paper on Causes of Cancer in Man and served as a rapporteur for a Dahlem Conference on Neoplastic Transformation: Mechanisms and Consequences, held on May 9-13, 1977.

Perugia: At the Sixth Quadrennial International Conference on Cancer in Perugia, which will concern Tumors of Early Life in Man and Animals, Dr. Mulvihill will travel at his own expense due to a shortage of government funds, to present observations on 1197 neoplasms in young domestic animals (under 2 years of age), using data from the Branch's Veterinary Medical Data Program.

Montreal: At the Fifth International Conference on Congenital Malformations, Dr. Miller will present an overview of transplacental environmental effects (cancer or malformations) in man. Just before the Conference, three teratology societies (U.S., European, and Japanese) will hold a joint-meeting at which Dr. Miller will speak as a representative of the Japanese Society on contributions to teratology by alert clinicians, and on the value of dried umbilical cords (traditionally stored for decades in Japan) in the study of certain past pollutants.

World Health Organization: At a Conference on Animal Models of Non-Mendelian Developmental Defects, held in Geneva on March 21-25, 1977, Dr. Priester served as Chairman and presented a paper based on data from the Veterinary Medical Data Program. Enroute he gave a seminar at the Royal Veterinary College in London to a gathering of veterinary and agricultural research units from southern England. He also visited the Royal Veterinary College in Copenhagen for discussions on a European data-processing system to replace the WHO system and collaborative studies, which are being phased out.

U.S.S.R.: Dr. Miller will serve as a member of the Fourth USA-USSR Meeting on Mammalian Somatic Cell Genetics Related to Neoplasia, which involves a 2-day symposium and visits to 6 cancer or genetics institutes in Moscow, Tbilisi, and Leningrad, June 5-17, 1977.

People's Republic of China: Under the sponsorship of the National Academy of Sciences in connection with the Committee on Scholarly Communication with the People's Republic of China, Dr. Miller will serve as a member of a delegation which will visit China for 3-4 weeks beginning on about September 18, 1977, for a series of binational discussions on clinical oncology (cause, prevention, diagnosis, and treatment).

All of the above foreign travel was funded from outside the Branch. The only travel to international meetings paid by the Branch was to Baltimore, where

Dr. Mulvihill participated in a UICC Workshop on Cancer Chromosome Data Banks, to New York for a meeting on multiple primary tumors (Drs. Li, Mulvihill, and Priester), and to Storrs, Connecticut, where Dr. Priester participated in an International Symposium on Pathobiology of Environmental Pollutants -- Animal Models and Wild Life as Monitors.

Veterinary Program:

In 1961 the Epizoology Section was initiated. At that time there was an absence of systematically collected data for epidemiologic research. The Section developed a procedure for making standard observations on a mass scale to permit machine-processing and retrieval. Fourteen veterinary colleges contribute about 15,000 abstracts per month summarizing diagnostic, demographic, and identifying information on each animal discharged. Each contributing institution receives a monthly listing of its data; NCI has the pooled data for its use. Since its beginning, the VMDP has accumulated more than 1.7 million abstracts, including 40,000 tumors reported during the period March 1964 through May 1977. Because the NCI file has become the largest single source of veterinary clinical data, the Epizoology Section has increasingly functioned as an information source for researchers of diverse interests and nationalities, including those in cancer research centers, the World Health Organization, and colleges of medicine and veterinary medicine.

The main theme for the Section this year has been refinement of all vocabularies, computer programs, Users Guides, and code books associated with the routine processing of data submitted to the Veterinary Medical Data Program, and documentation of special techniques used in analyzing VMDP data. Because we are turning over responsibility for data collection and file maintenance to a cooperative of VMDP participants at the end of the current fiscal year, it is thought crucial to permanent survival of VMDP that all supporting data should be in the very best condition possible before responsibility is transferred.

In addition, we have encouraged scientists outside of NIH to use the data. We have, for example, begun a report on similarities and differences in the occurrence of tumors of domestic animals reported to World Health Organization and to VMDP with pathologists at the veterinary colleges in Edinburgh and London. Cooperative studies have not been limited to cancer; we began preliminary studies on diseases in aged animals with scientists at Michigan State University.

Analytical Reviews and Other General Communications:

In an attempt to gain new perspectives and to familiarize oncologists with etiologic aspects of cancer, several categories of publications have been issued or are in preparation. Reivews or editorials were prepared on the history of the discovery of human teratogens, carcinogens, and mutagens, with lessons for the future; the relation between human carcinogens and teratogens; etiology of childhood cancer; causes of cancer in man; pollutants in breast milk; the bedside etiology of cancer; ethnic differences in cancer occurrence; host factors in human lung tumors; and the health implications of

water contaminants to man. The Childhood Cancer Etiology Newsletter, issued monthly by the Branch, is now in its fourth year. Also, a handbook on childhood cancer is being prepared for pediatricians to simplify information which should be helpful in discussing etiology, diagnosis, and therapy with parents.

In connection with a plan by the Director of NIH to increase awareness of NIH's contribution to the medical profession, the Branch was selected to prepare a pilot videotape on the bedside etiology of childhood cancer, for use in medical libraries. In addition, Dr. Mulvihill was interviewed about cancer families by the Voice of America.

Perhaps the major achievement of the year was publication of a volume, Genetics of Human Cancer, based on a conference sponsored by the Branch about 16 months before. The book contains papers and discussions on the relation between cancer and the genetics of germ cells (inheritance) and of body cells; the latter is widely thought to be a step in the development of environmentally induced cancer. We hope the book will stimulate interest especially at the clinical level in this neglected subject.

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28) Miller, R.W.: Bedside etiology of childhood cancer. Ca, a Journal for Clinicians. In press.

29) Miller, R.W.: Health implications of water contaminants in man. In Annals of The New York Academy of Sciences. In press.

30) Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F., Jr. (eds.): Genetics of Human Cancer. New York, Raven Press, 1977, 519 pp.

31) Mulvihill, J.J., Gralnick, H.R., Whang-Peng, J., and Leventhal, B.G.: Multiple childhood osteosarcoma in an American Indian family with erythroid macrocytosis and skeletal anomalies. Cancer. In press.

32) Mulvihill, J.J. and McKeen, E.A.: Genetics of multiple primary tumors: a clinical etiologic approach illustrated by three patients. Cancer. In press.

33) Mulvihill, J.J.: Genetic repertory of human neoplasia. In Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F., Jr. (eds.): Genetics of Human Cancer. New York, Raven Press, 1977, pp. 137-143.

34) Mulvihill, J.J.: Host factors in human lung tumors: an example of ecogenetics in oncology. J. Natl. Cancer Inst. 57: 3-7, 1976.

35) Mulvihill, J.J.: Host factors. In Harris, C. (ed.): Pathogenesis and Therapy of Lung Cancer. New York, Marcel Dekker, 1977. In press.

36) Mulvihill, J.J., Klimas, J.T., Stokes, D.C. and Risemberg, H.M.: Fetal alcohol syndrome: seven new cases. Am. J. Obstet. Gynecol. 125: 937-941, 1976.

37) Priester, W.A.: Canine intervertebral disc disease -- occurrence by age, breed, and sex among 8,117 cases. J. Theriogenology 6: 293-303, 1976.

38) Priester, W.A., Goodman, D.G. and Theilen, G.H.: Nine simultaneous primary tumors in a boxer dog. J. Am. Vet. Med. Assoc. 170: 823-826, 1977.

39) Priester, W.A.: Lymphomas and animal-protein consumption. (Letter) Lancet 1: 201-202, 1977.

40) Priester, W.A., Wade, W.M. and McKay, F.W.: 1977 Coding Supplement to Standard Nomenclature of Veterinary Diseases and Operations. DHEW Publication No (NIH) 77-1253. USDHEW: PHS, NIH, NCI. Wash., D.C., U.S. Govt. Print. Off., 1977, 417 pp.

41) Priester, W.A.: Multiple primary tumors in domestic animals: a preliminary view with particular emphasis on tumors in dogs. Cancer. 1977. In press.

42) Roseman, B. and Mulvihill, J.J.: Melkersson-Rosenthal syndrome in childhood. Pediatrics. In press.

43) Stokes, D.C., Mulvihill, J.J. and Zinkham, W.H.: Acute lymphoblastic leukemia in sporadic neurofibromatosis. (Letter) Am. J. Dis. Child. In press.

(44) Safyer, A.W. and Miller, R.W.: Childhood cancer: etiologic clues from epidemiology. J. School Health: 158-164, 1977.

(45) Dorn, C.R. and Priester, W.A.: Epidemiologic analysis of oral and pharyngeal cancer in dogs, cats, horses, and cattle, J. Am. Vet. Med. Assoc. 169: 1202-1206, 1977.

(46) Miller, R.W.: Pollutants in breast milk. J. Pediatr. 90: 510-512, 1977.

(47) Miller, R.W. and Flynt, J.W., Jr.: Epidemiological surveillance. Fetal and Perinatal Diseases: 287-293, 1977.

(48) Miller, R.W.: The alert practitioner as a cancer etiologist. In Proceedings of Environmental Carcinogenesis Conference. Houston, Texas, Jan. 12-13, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04325-15 CEB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Planning and Development in Cancer Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	R.W. Miller, M.D.	Chief, Clinical Epidemiology Branch	CEB	NCI
OTHER:	W.A. Priester, M.D.	Head, Epizoology Section	CEB	NCI
	Frederick P. Li, M.D.	Head, Clinical Studies Section	CEB	NCI
	J.J. Mulvihill, M.D.	Head, Clinical Genetics Section	CEB	NCI
	G.W. Beebe, Ph.D.	Expert, Biostatistics	CEB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Field Studies and Statistics, DCCP

INSTITUTE AND LOCATION

NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this undertaking are to originate new epidemiologic approaches to the study of cancer causation in man and animals, to develop sources of data related to specific epidemiologic problems, to stimulate epidemiologic research in other health agencies and to develop an on-the-job training program for medical and statistical officers.

Opportunities for research in cancer epidemiology are based on questions arising from clinical or laboratory observations, on unusal groupings of cancer cases in the population, and on study of the characteristics of groups of persons prior to the onset of a specific type of cancer as compared with similar persons who have not developed the disease. In parallel with this, plans are made for on-the-job training in cancer epidemiology for research physicians, veterinarians, and statisticians.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.

2. To develop sources of data related to specific epidemiologic problems.

3. To stimulate epidemiologic research in other health agencies.

4. To develop on-the-job training for medical and statistical officers.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.

2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples -- clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons (examples -- clinical health-surveys, and military medical examinations).

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the

usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

We plan to continue and extend etiological studies of patients with cancer and of cancer families at NIH or elsewhere in the city or nation, and to encourage similar approaches internationally. Specimens from cases of interest will be obtained for laboratory studies using new procedures in an attempt to develop further understanding of factors that increase host-susceptibility to environmental carcinogens. New ways of portraying cancer mortality data will be undertaken to seek peculiarities in distribution which have etiological implications. Tests of hypotheses will be made as they arise from experimental or clinical observations. Studies of data from about 2 million domestic animals should continue to reveal similiarities or differences in the occurrence of cancer and other diseases in comparisons of various species, including man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04371-13 CEB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

National Cancer Institute Veterinary Medical Data Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI : W. A. Priester Head, Epizoology Section CEB NCI
OTHER: F. W. McKay CEB NCI

COOPERATING UNITS (if any) Schools and Colleges of Veterinary Medicine: Michigan State, Missouri, Minnesota, Iowa State, Ontario, Purdue, Georgia, Kansas State, Illinois, California, Ohio State, Saskatchewan, Colorado State, Auburn, Texas. Also see Project Report Z01 CP 04380-05 CEB

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Field Studies and Statistics Section, DCCP

INSTITUTE AND LOCATION

NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.40	.40	2.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to provide animal disease data as a research resource, an abstract of clinical findings resulting from each patient discharge at participating institutions is sent to Veterinary Medical Data Program central processing. Abstracts are edited, reports are prepared for participants and information is stored for use in current and future epidemiologic study. The data bank, which included information on 1.6 million patients through December 1976, is often used as a reference source by scientists at NCI and elsewhere.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed:

Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizoology Section.

Major Findings:

Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from 13 veterinary colleges in this country and 2 in Canada. The program has been endorsed and implemented in Europe under the auspices of WHO. For the first time, veterinary medicine and the medical sciences have benefit of a national and international animal disease data system. A number of cooperative studies were begun during the year with faculty members at the various participating colleges of veterinary medicine.

Significance to Bio-medical Research and the Program of the Institute:

The comparative (veterinary) medical data retrieval system instituted at the College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage, and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to 14 additional veterinary colleges. Establishment and maintenance of reliable sources of veterinary medical data provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, congenital defects, and gerontological diseases. Continued NCI Epidemiology Branch animal studies research function depends upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease. The data bank, which included information on more than 1.5 million patients through 1976, is often used as a reference source by scientists at NCI and elsewhere.

Proposed Course of Project:

Responsibility for all aspects of quality control and routine processing will be transferred to a cooperative of current participants in the coming year. We expect that data collected will be available to us on a cost-reimbursable basis.

Project No. Z01 CP 04371-13 CEB

Date initiated: October 22, 1965

Current annual level: $66,000

Publications

Priester, W.A.: Canine intervertebral disc disease -- Occurrence by age, breed, and sex among 8,117 cases. J. Theriogenology 6: 293-303, 1976.

Priester, W.A., Wade, W.M. and McKay, F.W.: 1977 Coding Supplement to Standard Nomenclature of Veterinary Diseases and Operations. DHEW Publ. No. (NIH) 77-1253. Washington, D.C., U.S. Govt. Print. Off., 1977, 417 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04377-07 CEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Familial, Congenital and Genetic Factors in Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	J.J. Mulvihill, M.D.	Head, Clinical Genetics Section	CEB	NCI
OTHER:	R.W. Miller, M.D.	Chief	CEB	NCI
	F.P. Li, M.D.	Head, Clinical Studies Section	CEB	NCI
	A.W. Safyer		CEB	NCI
	E.A. McKeen, M.D.		CEB	NCI
	D.M. Parry, Ph.D.		CEB	NCI

COOPERATING UNITS (if any)
Environmental Epidemiology Branch and various other NCI clinical and experimental groups

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Field Studies and Statistics Section, DCCP

INSTITUTE AND LOCATION
NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	3.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Study of genetic diseases with neoplastic manifestations and detailed investigation of families at high risk of cancer may help detect environmental and genetic influences in carcinogenesis.

Methods include review of prior medical and environmental history; documentation of history; collection and distribution of biological specimens; review of hospital records of patients with selected genetic diseases or neoplasms; and survey of childhood cancer death certificates in the U.S. since 1960.

Report on multiple malignancies emphasized the co-existence of additional benign tumors in patients, as well as benign and malignant tumors in their families. Laboratory studies revealed subclinical immune defects in a family with EBV-related malignancies and a new syndrome of birth defects, and minor bony birth defects and bone marrow dysfunction in a family with osteosarcoma. A monograph, Genetics of Human Cancer, was edited and published. The same approach will be continued, using new laboratory methods and epidemiologic clues from other sources as available.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To document the occurrence of patterns of familial aggregations of neoplasms; to study selected families by genetic and laboratory investigations, in an effort to elucidate the degree to which heredity and the common familial environment contribute to the etiology of neoplasms. To distribute tissue and blood samples from family members to interested laboratory investigators for etiologic study by biochemical, cytogenetic, immunologic, viral, and tissue culture methods. To study similarly patients with birth defects and other hereditable disorders that may predispose to malignancy. To identify genetic factors and disorders associated with cancer.

Methods Employed:

Interview of patients with cancer or other diseases with respect to familial occurrences especially of cancer and birth defects, as well as prior medical and environmental history; documentation of history by obtaining appropriate vital records and hospital charts; collection and distribution of biological specimens from such families; review of hospital records of series of patients with selected congenital and genetic diseases or neoplasms; survey of childhood cancer death certificates in the U.S. since 1960 to identify sib aggregation.

Major Findings:

A number of families has been studied with cancers of single sites and of multiple sites. In a family with Epstein-Barr virus-related malignancies and a new pattern of birth defects, immunological abnormalities were discovered in affected individuals and some clinically normal relatives. In a family with osteosarcoma, immune function was normal but bone formations and defective hematopoiesis were discovered, suggesting a new osseous syndrome of cancer and birth defects. In a separate study, 3 patients had 19 malignancies, as well as an array of benign neoplasms; the same benign and malignant tumors were seen among their relatives.

Case reports were prepared on patients with neurofibromatosis and acute lymphoblastic leukemia, a Waardenberg-like syndrome and osteosarcoma, along with separate series of patients with craniopharyngioma and fetal alcohol syndrome.

A monograph, Genetics of Human Cancer, was edited and published, as well as individual reviews on Mendelian traits predisposing to malignancy, host factors in human lung cancer, and irradiation teratogenesis.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications,

enabling surveillance and early diagnosis of neoplasms.

Proposed Course of Project:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as available.

Publications

Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F., Jr. (eds.): Genetics of Human Cancer. New York, Raven Press, 1977, 519 pp.

Mulvihill, J.J., Gralnick, H.R., Whang-Peng, J. and Leventhal, B.G.: Multiple childhood osteosarcoma in an American Indian family with erythroid macrocytosis and skeletal anomalies. Cancer. In press.

Mulvihill, J.J. and McKeen, E.A.: Genetics of multiple primary tumors: a clinical etiologic approach illustrated by three patients. Cancer. In press.

Mulvihill, J.J.: Genetic repertory of human neoplasia. In Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F., Jr. (eds.): Genetics of Human Cancer. New York, Raven Press, 1977, pp. 137-143.

Mulvihill, J.J.: Host factors in human lung tumors: an example of ecogenetics in oncology. J. Natl. Cancer Inst. 57: 3-7, 1976.

Mulvihill, J.J.: Host factors. In Harris, C. (ed.): Pathogenesis and Therapy of Lung Cancer. New York, Marcel Dekker, 1977. In press.

Stokes, D.C., Mulvihill, J.J. and Zinkham, W.H.: Acute lymphoblastic leukemia in sporadic neurofibromatosis. (Letter) Am. J. Dis. Child. In press.

Meyer, W.J., Udvarhelyi, G.W., Clopper, R.R., Aarabi, B. and Mulvihill, J.J.: Endocrine evaluation of craniopharyngioma. J. Neurosurg. In press.

Clopper, R.R., Meyer, W.J., Udvarhelyi, G.B., Money, J., Mulvihill, J.J. and Piasio, M.: Postsurgical IQ and behavioral data on twenty patients with a history of childhood craniopharyngioma. Psychoneuroendocrinology. In press.

Mulvihill, J.J., Klimas, J.T., Stokes, D.C. and Risemberg, H.M.: Fetal alcohol syndrome: seven new cases. Am. J. Obstet. Gynecol. 125: 937-941, 1976.

Roseman, B. and Mulvihill, J.J.: Melkersson-Rosenthal syndrome in childhood. Pediatrics. In press.

Miller, R.W. and Mulvihill, J.J.: Teratogen update: small head size after atomic irradiation. Teratology 13: 345-348, 1976.

Chesney, P.J., Teets, K.C., Mulvihill, J.J., Salit, I.E. and Marks, M.I.: Successful treatment of Candida meningitis with amphotericin B and 5-fluorocytosine in combination. J. Pediatr. 89: 1017-1019, 1976.

Blattner, W.A., Lubiniecki, A.S., Mulvihill, J.J. and Fraumeni, J.F., Jr.: A single gene does not control SV-40 T-antigen expression in cancer families. Proc. Am. Assn. Cancer Res. 18: 63, 1977.

Parry, D.M., Safyer, A.W. and Mulvihill, J.J.: A Waardenburg-like syndrome with osteosarcoma, ectodermal dysplasia, joint abnormalities and hypogonadism. The 1977 Birth Defects Conference. June 8-10, 1977.

McKeen, E.A. and Mulvihill, J.J.: A possibly X-linked malformation-retardation syndrome segregating independently of diverse malignancy. 5th International Conference on Birth Defects. August 21-27, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04380-07 CEB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of the zoographic characteristics of domestic animals with tumors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI : W.A. Priester Head, Epizoology Section CEB NCI
OTHER: J.J. Mulvihill Head, Clinical Genetics CEB NCI
F.W. McKay CEB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Field Studies and Statistics, DCCP

INSTITUTE AND LOCATION

NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.40	.40	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using data from the 32,000 tumors (800,000 patient-years-at-risk) reported to the Veterinary Medical Data Program through December 1974, we expect to provide a reference of the distribution of spontaneous tumors in domestic animals. Further, using this relatively large series, we intend to search for unusual zoographic distributions of tumors, which may suggest possible clues regarding the etiology of cancer. The patient characteristics we expect to emphasize are age, breed/species, sex; the tumor characteristics are degree of malignancy, site and histogenic type. Only the bovine, equine, feline and canine species had a sufficient number of tumors reported to be of value in analytic studies. Currently conducting studies on multiple primary tumors, hemangioendotheliomas, and comparison of tumor occurrence reported by North American Veterinary Colleges with occurrence in Europe (reported to WHO).

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To provide a reference of the distribution of spontaneous tumors in domestic animals. To search for unusual distribution of tumors, within a large, animal hospital population, which might suggest possible clues regarding the etiology of cancer.

Methods Employed:

Using patients with tumors reported to the Veterinary Medical Data Program as case series, and all patients seen during like periods as population references, the following will be studied:

Characteristics of patients:	age, breed/species, sex
Characteristics of tumors	:	degree of malignancy, site, and histogenic type.

In addition, other factors may be studied as the analyses progress.

Major Findings:

32,000 tumors were reported during the period March 1964 through December 1975 among 800,000 animal-years-at-risk. The proportion of patients with tumors was highest among dogs and lowest among horses, when the four major species categories, bovine, equine, feline, and canine, were considered. In almost all of the studies of specific tumors conducted so far age has been generally associated with an increasing risk for tumors but not to the same degree for each specific tumor type. Invariably, one or more breeds of dogs can be identified as being at excess risk for the specific tumor type under study. While no striking associations between specific tumor type and other primary tumors in the same patient have been noted, several associations were identified that seemed similar to conditions described for man.

Significance to Bio-medical Research and the Program of the Institute:

Animal studies done by the Branch have demonstrated several relationships of interest in cancer research -- bone sarcoma and size, canine lymphoma and certain breeds, canine mastocytoma and breed ancestry. This study is designed to detect high risk groups in a systematic fashion. The results may well define animal subgroups suitable for studies of cancer etiology that would be impractical in a human population.

Proposed Course of Study:

Data will be added to the tumor file and reference population base at the end of each fiscal year. While some high-frequency tumors will be studied, the main effort will be directed towards study of those spontaneous tumors in domestic animals that have been so infrequently reported in the literature that characterization of patients with these tumors has never been done.

Date initiated: May 1970

Current annual level: $31,000

Publications

Dorn, C.R. and Priester, W.A.: Epidemiologic analysis of oral and pharyngeal cancer in dogs, cats, horses, and cattle. J. Am. Vet. Med. Assoc. 169: 1202-1206, 1976.

Priester, W.A., Goodman, D.G. and Theilen, G.H.: Nine simultaneous primary tumors in a boxer dog. J. Am. Vet. Med. Assoc. 170: 823-826, 1977.

Priester, W.A.: Multiple primary tumors in domestic animals: a preliminary view with particular emphasis on tumors in dogs. Cancer. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04400-17 CEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Studies Section, Boston, Massachusetts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Frederick P. Li, M.D. Head, Clinical Studies Section CEB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Field Studies and Statistics, DCCP

INSTITUTE AND LOCATION
NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Clinical Studies Section provides the Clinical Epidemiology Branch with a large patient resource for bedside studies of cancer etiology. In the last 3 years, over 400 questionnaire interviews of childhood cancer patients have been completed, and an analysis of findings is in progress. Through the interviews, several unusual family aggregates of cancer have been identified for more detailed clinical and laboratory studies. Additional familial clusters of cancer, including childhood cancer in 38 sets of siblings, have been identified through retrospective review of hospital records at several Boston medical centers.

A Registry of Survivors of Childhood Cancer has been set up through collaboration of the Clinical Studies Section and the Sidney Farber Cancer Center. The 500 registrants represent approximately 2% of all survivors of childhood cancer in the last 2 decades. Patients in the series are being observed for second malignancies, cancer in close relatives, and genetic effects in progeny.

In a new study, the unusually high mortality of bladder cancer in Vermont, and of lung cancer in selected Maine counties are being investigated through an analysis of death certificates.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1) To study through clinical observation at the bedside causes of human cancers.

2) To identify genetic factors in the development of human cancers.

3) To study the sequelae of childhood cancers in successfully treated patients, and in their progeny.

4) To conduct collaborative epidemiologic studies of human cancers utilizing the clinical resources available in Boston and New England regions.

Methods Employed:

Each child admitted for cancer therapy at the Sidney Farber Cancer Institute is examined for clues to etiology of the neoplasm. When exceptional clinical observations are made, appropriate follow-up epidemiologic and laboratory investigations are conducted. To date, 400 interviews have been completed, and are under analysis.

A registry has been established of 424 patients who have survived childhood cancer for at least 5 years. These patients are being studied to determine the probability of cure of childhood cancer, and the somatic and genetic effects of the disease.

Several striking family aggregates of specific cancers have been identified. Family members are under study to identify reasons for the susceptibility, and to detect early cancers.

Major Findings:

1) An apparently new genetic syndrome has been identified in a family with acute leukemia, hypoplasia of the marrow and the cerebellum, and a C-chromosome deletion.

2) Several families have been identified with aplastic anemia affecting multiple generations; this finding has not been reported previously.

3) A family was found to have renal cell carcinoma affecting multiple relatives. Screening of asymptomatic family members has identified one additional case of the disease. Laboratory studies have shown a familial balanced chromosomal translocation that may have a pathogenetic role in the cancers.

4) A life-table analysis of 5-year survivors of childhood cancers shows an 80% probability of survival for an additional 20 years.

Significance to Bio-medical Research and the Program of the Institute:

The studies of the Clinical Studies Section identify unusual clinical observations for more detailed investigations by epidemiologists and laboratory investigators. Also, the relevance to humans of studies of laboratory animals is examined through clinical observations of patients at the bedside.

Proposed Course of Project:

The Clinical Studies Section will continue with established studies of childhood cancers. These projects will examine the etiologic role of genetic factors and pre-natal exposures, and the late effects of these diseases. In addition, the methods that have proved useful in childhood cancer studies will be applied to study appropriate cancers in adults. These studies will examine family aggregates of cancer, and the epidemiologic features of rare and seldom studied forms of adult malignancies.

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)
Fiscal Year 1977
Animal Studies

VETERINARY MEDICAL DATA PROGRAM

Purchase orders between the Epizoology Section and Colleges of Veterinary Medicine at the University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, Kansas State University, Ohio State University, University of Saskatchewan, Purdue University, University of Minnesota, University of California, Colorado State University, Auburn University, and Texas A & M University.

Title: Purchase Veterinary Case Data.

Objectives: To provide reliable data on disease in domestic animals to Epizoology Section and to further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: One year. Emphasis will be directed towards transfer of responsibility for quality control and for preparation of machine-readable data to participants.

Date Initiated:

Michigan State University	-	November 1965
Iowa State University	-	October 1965
Ontario Veterinary College	-	July 1966
University of Georgia	-	January 1967
University of Missouri	-	February 1967
University of Minnesota	-	December 1967
University of Illinois	-	January 1968
Kansas State University	-	January 1968
Purdue University	-	January 1968
Ohio State University	-	January 1969
University of Saskatchewan	-	January 1969
University of California	-	July 1970
Colorado State University	-	August 1972

Auburn University	-	November 1974
Texas A & M University	-	September 1976

Current Annual Level: $66,000

CONTRACT NARRATIVE
CLINICAL EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1977

Clinical Genetics Section

BIO TECH RESEARCH LABORATORIES, INC. (NO1-CP-61011)

Title: Cytogenetic evaluation of high risk cancer families

Contractor's Project Officers: Chang-Chin Tai, Ph.D.

Project Officer(NCI): John J. Mulvihill, M.D.

Objectives: To determine if persons prone to cancer because of their personal or family history have cytogenetic abnormalities.

Methods Employed: Standard karyotypic techniques, including differential staining for banding, are applied to biologic specimens from individuals at high risk for cancer under study by the Clinical and Environmental Epidemiology Branches. Specimens are lymphocytes from peripheral blood and fibroblast cultures. Dr. J. Whang-Peng,NCI, and C. C. Huang serve as consultants.

Major Findings: Over 250 specimens have been accessioned. The observation of chromosomal breaks in fibroblasts validates the presumptive diagnosis of Fanconi anemia and certain aneuploidies and permits meaningful interpretations of further studies on the cells.

Significance to NCI Program and Biomedical Research: The Epidemiology Branches search for genetic markers of human cancer by combining critical clinical and epidemiologic evaluation with a battery of modern laboratory approaches, one of which is provided by this contract. Several human malignancies are well known to be associated with cytogenetic abnormalities that may have etiologic, diagnostic, therapeutic, and prognostic implications. The contract provides a means for systematic cytogenetic investigations of persons or families at high risk of cancer.

Proposed Course of Project: Specimens will continue to be accessioned. Priority of testing will be determined to expedite the potentially most informative specimens submitted by us and various NCI collaborators. Results will be interpreted in the context of our clinical-epidemiologic evaluation and other laboratory parameters of immunologic, genetic, and virologic nature.

Date Current Contract Initiated: September 23, 1975

Current Annual Level: $28,336

ANNUAL REPORT OF THE ENVIRONMENTAL EPIDEMIOLOGY BRANCH
NATIONAL CANCER INSTITUTE
July 1, 1976 through September 30, 1977

This is the second report of the Environmental Epidemiology Branch which was created in December 1975. The objective of the Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources best available at the national level. The program expanded considerably this year when 17 new positions were allocated to the Branch, permitting it to double in size. The following appointments were made: Pierre Decoufle, Sc.D., a biostatistician with a background in occupational health; Charles E. Land, Ph.D., a biostatistician with experience in radiation studies; B. J. Stone, Ph.D., a mathematician and computer analyst. William A. Blattner, M.D., returned to the Branch after a one-year assignment with the NCI Medicine Branch, and Ronald R. Reimer, M.D., arrived on a one-year detail from the Medicine Branch. Aaron E. Blair, Ph.D., an epidemiologist; and Linda W. Pickle, Ph.D., a biostatistician, became Staff Fellows. Kenneth J. Rothman, Dr.P.H., an epidemiologist from Harvard University, and Daniel J. Grauman, M.A., a biostatistician from State University of New York in Buffalo, joined the Branch as part of the Intergovernmental Personnel Act. Kenneth P. Cantor, Ph.D., an environmental epidemiologist, extended his assignment on detail from the Environmental Protection Agency (EPA). Takashi Yanagawa, D.Sc., a Japanese biostatistician, became a Visiting Scientist. Boyd L. Stephenson and Richard I. Ramsbottom joined the Branch as computer specialists, and Louise A. Brinton, M.P.H., became an epidemiological research assistant. Terry L. Thomas, a sociologist in the Branch, received an M.S. in biostatistics at Georgetown University, and Nancy A. Dalager, a computer programmer, is completing her requirements for the same degree. Soon to arrive are four epidemiologists: John D. Boice, Jr., Sc.D., with experience in the area of radiogenic cancer; Kiyohiko Mabuchi, M.D., Dr.P.H., with a broad background in cancer studies; Shinkan Tokudome, M.D., with experience in occupational cancer; and Mark H. Greene, M.D., returning after a one-year detail to the Medicine Branch. In addition, three health statisticians will soon join the staff: Patricia A. Hartge, M.A., M.S., Linda E. Morris, M.P.H., and Linda S. Mendelsohn, M.P.H.

RESEARCH PROGRAM

Geographic Patterns

Mapping--In the past, alert clinicians have been the primary means of identifying case clusters of cancer, which have led often to the recognition of environmental carcinogens. These discoveries were usually possible because the particular tumors seen were ordinarily rare in the general population. To provide a systematic means for identifying geographic variation and clustering, particularly with respect to more common tumors, the Branch has analyzed U.S. cancer mortality on a county level and prepared computer-

generated color maps for 35 cancer sites. In 1975 an atlas was published for the white population, and this year a companion atlas for the non-white population was released (1). The maps have revealed a surprising number and variety of geographic patterns and clusters, providing a series of new etiologic clues which can now be pursued by more definitive studies. In preparation are maps of non-neoplastic diseases with special emphasis on those that may be etiologically related to cancer. The clues derived from the cancer maps are being pursued actively by the Branch, using a step-wise approach of correlation and analytic studies summarized in review articles (2-5).

Correlation studies--To seek explanations for the geographic variation in cancer across the United States, a series of correlation analyses were undertaken this year, linking the county mortality rates with demographic and environmental data available at the county level. For some studies the starting point is a particular environmental exposure. For example, in counties with petroleum industries, the rates were significantly high for cancers of the lung, nasal cavity, melanoma and other skin cancers (6). In furniture-industry counties, the risk was greatest for cancer of the nasal cavity (7). The exposure studied may be non-occupational as well, affecting the general community. For instance, no excess of cancer could be found in counties where the drinking water was naturally or artificially fluoridated (8), despite well-publicized reports to the contrary by a private agency. When cancer mortality rates were correlated with the levels of halogenated hydrocarbons in drinking water according to EPA surveys, a relationship was found primarily to bladder cancer (9), which is consistent with the results of several recent studies. Although asbestos fibers have been present in high levels in the drinking water of Duluth, Minnesota, the patterns of cancer mortality and morbidity in that area have not shown any peculiarities (10). Epidemiologic methods for evaluating the carcinogenic effects of water-borne pollutants have been reviewed (11,12).

In other correlation studies, the starting point has been a particular cancer site. For example, large bowel cancer showed a striking north-south differential in both sexes, with high rates throughout the northeast and low rates in the south (13). Mortality was consistantly elevated in counties with large populations, higher socioeconomic class, and high percentages of residents of Irish, German, or Czechoslovak descent. However, these demographic factors only partly explained the northern predominance of this tumor. A survey was made of the limited data available on dietary habits by region and on alcohol sales by county, but the concomitant variation with bowel cancer rates was not impressive. Lung cancer was also highlighted by elevated mortality in the northeast and around large metropolitan areas, but in white males the highest rates were clustered in contiguous rural counties along the Gulf of Mexico, particularly in Louisiana, and along the southeast Atlantic coast (14). Mortality was elevated in counties with paper, chemical, petroleum, and transportation (shipbuilding) industries. The industrial correlations were not attributed to demographic or other manufacturing operations, and may account for at least part of the high risk of lung cancer in southern coastal counties. Cancer of the oral cavity also

showed excessive rates among males in urban areas throughout the country, but among females the tumor predominated in the rural south (15). It seems likely that the urban gradient in males results from alcohol beverage consumption and tobacco smoking, while the southern excess in females is related primarily to oral snuff use. Mortality from oral cancer was elevated further in counties with leather, paper, and chemical manufacturing industries (in males), and with textile and apparel industries (in females). The industrial correlations were independent of demographic variables, but snuff use by southern women in the textile and apparel industries may have influenced this association. The patterns for esophageal cancer were similar, with higher rates among males in the northeast and among females in the south (16). There was a strong urbanization gradient among white males and non-whites of both sexes, and a 2:1 excess among blacks, especially in urban areas. However, exceptionally high rates were clustered among blacks in a narrow band of rural counties along the southeast Atlantic coast, particularly around Charleston, South Carolina. The patterns for other tumors arising in the head and neck region were also reviewed (17). Finally, the distribution of breast cancer was found to parallel in many ways that for large bowel cancer. However, the northern predominance of this tumor was primarily among postmenopausal women, while mortality among premenopausal women was distributed almost uniformly across the country (18). Socioeconomic status, German ethnicity, and colon cancer mortality were strong indicators of the rates for postmenopausal women, but only partly explained the northern excess and latitudinal gradient. In contrast, fertility patterns and ovarian cancer mortality were more closely linked to breast cancer among premenopausal women. The geographic peculiarities of this tumor in older women suggest extrinsic risk factors that remain to be identified, while the patterns in the younger women point to the primary role of reproductive and genetic determinants.

Death certificate analyses--As a quick and inexpensive preliminary step to the field studies, examination is made of death certificates in certain communities with high rates for a particular cancer or with an exposure of interest. Statements on occupation, place of birth, and duration of residence may be compared among cancer cases and controls in several areas of the country. In this way a four-fold excess risk of nasal cancer was found among furniture workers in North Carolina counties where that industry is heavily concentrated (19). A link between woodworking and nasal cancer has been demonstrated in several nations, but this is the first indication of a similar hazard in the United States. Another study involved death records for lung cancer in coastal Georgia, and showed higher frequencies of construction, wood and paper, and chemical workers among cases than controls. The preliminary tabulations of lung cancer records from northern Florida, however, revealed only a small increased risk among workers in pulp and paper mills. Certificates are being examined or requested from other high-risk communities, including oral cancer in Missouri and North Carolina; liver and gallbladder cancers in New York, West Virginia, Minnesota, Ohio, Michigan, Wisconsin, and Texas; bladder cancer in New Jersey, Vermont, Louisiana, Wisconsin, Illinois, and Iowa; lung cancer in Maine and Louisiana; nasal and nasopharynx cancers in North Carolina and Louisiana; pancreas cancer in Louisiana; and colon cancer in Nebraska. The studies in New Jersey

and Louisiana, which have excessive rates for several cancers, are enhanced by collaborative arrangements with the New Jersey State Department of Health and Tulane University.

Field studies--The final step in our approach is the testing of specific hypotheses by analytic field studies in high-risk communities, conducted with the assistance of a support services contract with Westat, Inc. The first major investigation has been a case-control interview study of lung cancer patients among residents along the southeast Atlantic Coast. In collaboration with the Center for Disease Control and the Georgia Department of Human Resources, incident lung cancer cases (1970-76) and controls were identified from hospital records in the coastal Georgia city of Brunswick. Interviews of approximately 150 cases and 300 controls (or their next of kin) for lifetime smoking, occupation and residence histories have been completed. Local cooperation was excellent, with the field team achieving a participation rate of 98% and only ten interview refusals. The team then moved to Savannah where lung cancer cases and matched controls have been identified for interview. A study is planned also for Jacksonville, Florida, to help evaluate the role of occupational and other risk factors responsible for the excess lung cancer in the southeastern part of the country.

A similar project is getting underway in Butler and Colfax counties in eastern Nebraska. Despite the northeastern predominance of colon cancer in the United States, these contiguous farming counties in Nebraska have very high rates for this tumor in both sexes. In collaboration with Creighton University, interview studies of incident cases and controls will seek to identify dietary, other environmental, and genetic determinants of this geographic cluster. Also planned for this year are case-control studies of oral cancer in North Carolina; bladder cancer in Michigan, Vermont, and New Jersey; and lung cancer in Maine. Contact has been made with the EPA for collaborative studies in certain areas where epidemiologic findings may be correlated with available measurements on air, water and other pollutants, or where new lines of environmental investigation appear warranted. From the experience of the Branch to date, the geographic patterns of cancer have proven to be useful in providing clues to risk factors and directing attention to communities where further epidemiologic study should be concentrated in the search for environmental carcinogens.

Ethnic Differences

The U.S. cancer mortality resource has been utilized also to uncover racial and ethnic differences in cancer risk. Early reports from the Branch emphasized white-nonwhite differences, and the experience of Chinese-Americans, American Indians, and Alaskan natives. The atlas on geographic patterns for the nonwhite population (1) permitted a comparison of age-adjusted and age-specific mortality rates for each racial group in the United States. Compared to other nonwhite groups, mortality among blacks was high for cancers of the mouth and throat, esophagus, stomach, pancreas, larynx, lung, bladder, cervix, and multiple myeloma. American Indians had increased mortality from cancers of the biliary passages and liver (primarily gallbladder in females) with lesser elevations for cancers of nasopharynx, testis, cervix,

kidney, and thyroid (in females). Mortality in Chinese was very high for cancer of the nasopharynx, but increases were seen also for cancers of the esophagus, large bowel, biliary passages and liver (primarily liver in males), lung, and thyroid. Japanese experienced excessive mortality from stomach cancer, and to a lesser extent pancreas cancer (in males).

A mortality study of cancer among Alaskan natives, reported by the Branch in 1975, prompted an incidence survey covering a five-year period (20). There were fewer cancer cases overall than expected relative to U.S. rates, but the risk was significantly increased for certain sites: the nasopharynx in both sexes, liver in males, and salivary glands, gallbladder, kidney, and thyroid in females. Compared with earlier reports, the incidence figures suggest marked changes in cancer risk among Alaskan natives over the past two decades, with declines in esophageal and cervical cancers, and increases in cancers of the lung and large bowel. Field studies are being conducted on nasopharyngeal and other cancers in Alaska that may help identify etiologic factors.

To clarify racial differences for nasopharyngeal cancer in the United States, a study was made of death certificates and incidence statistics that yield cell-type data (21). Three age peaks were identified, with racial and epidemiologic distinctions that may reflect different etiologies. There was a four-fold excess risk of sarcomas in white children under age 10, and a four- to seven-fold excess of carcinomas in teenage blacks. Mortality in young people was greater in the South, with the excess in blacks linked to rural residence and low socioeconomic status. The characteristics of nasopharyngeal carcinoma under age 25 suggest the role of environmental (infectious?) agents, and contrast with the pattern of occurrence after age 25 that includes a predominance of Chinese-Americans.

Drugs

Drugs have been generally thought to contribute only slightly to the overall risk of cancer, but recent evidence suggests that the hazard may be much greater than anticipated. This year a number of projects were undertaken to evaluate the carcinogenic effects of various drugs.

Estrogens--In collaboration with Harvard University, one Branch member conducted a follow-up study of 1,891 women receiving conjugated estrogens at menopause for an average of 9 1/2 years (22,23). Cancer of the endometrium occurred two to three times more frequently than expected, and showed a dose-response relation with strength of tablet and number of years of use. Other relationships appeared that call for further study. Breast cancer showed an overall 30% excess, which increased with duration of follow-up and reached two-fold after ten years. In addition, ovarian cancer occurred two to three times as often as expected, yet there was no evidence of a dose-response relation and the excess seemed limited to a small group of women also using the synthetic estrogen, diethylstilbestrol. The number of patients with cancers other than breast and gynecologic organs was about one-half that expected, due mainly to a deficiency of large bowel cancer. However, there was no indication of a dose-response relation between estrogen

use and "protection" against this tumor, and the possible underascertainment of non-gynecologic cancers from the records of a gynecologic practice could not be ruled out.

The relationship between conjugated estrogens and endometrial cancer was confirmed by a case-control study of 205 women with this tumor (24). Compared to women with hysterectomy for benign disease, the relative risk associated with conjugated estrogens was 3.1. Users of other forms of systemic estrogens showed similar elevations in risk. The relative risk was related to duration of use, progressing from no evidence of risk among those using the hormone under five years, to an 11.5-fold greater risk for those using it for ten years of more. Risk was also related to the strength of medication, being 12.7 for users of the 1.25-mg. tablets as compared to a two- to four-fold greater risk among users of lesser strength tablets.

The possibility that synthetic forms of estrogen produce endometrial cancer was indicated by a series from the NCI End Results Program of 45,853 women with breast cancer diagnosed after age 49 (25). There was an excess risk for cancer of the uterine corpus, primarily among women receiving hormones, and the risk tended to rise with increasing interval from first treatment.

Recent evidence suggests that oral contraceptives may cause serious illness, including liver adenoma and possibly endometrial cancer. From a follow-up study of women taking oral contraceptives, conducted in collaboration with Harvard University, use of these agents was found to vary with social and reproductive characteristics that are risk indicators for many diseases (26). There was an increased risk of hospitalization for thromboembolic disease (relative risk = 1.5), mental illness, hyperthyroidism, hypertension, and cancer of the cervix. Users were hospitalized for many non-life threatening conditions 20 to 40% more frequently than were non-users.

To further evaluate the carcinogenic effect of estrogenic compounds and other drugs, several case-control investigations are being carried out, including studies of endometrial cancer in collaboration with Johns Hopkins University and breast cancer with the Kaiser Health Plan in Portland, Oregon.

Alkylating agents--An increased risk of leukemia has been reported in patients with multiple myeloma and Hodgkin's disease treated with alkylating agents, but it has been difficult to exclude the possibility that longer survival in these hematopoietic disorders may permit expression of an intrinsic predisposition to leukemic transformation. To estimate the leukemogenic potential of these agents, several institutions using these drugs in advanced ovarian cancer were surveyed for second primary neoplasms (27). Thirteen cases of acute nonlymphocytic leukemia (ANLL) occurred in a total of 5,455 patients. All thirteen had received alkylating agents, with nine also given radiotherapy. The relative risk for patients given chemotherapy was 36 and rose to 171 for those surviving two years. To evaluate the role of therapy versus underlying disease, an historical control of 13,309 ovarian cancer patients in the End Result Program was analyzed, but no excess of leukemia was noted, even among 6,596 women receiving radiation.

Thus, the excess of ANLL in the multi-hospital survey appeared attributable to alkylating agents, with the effect possibly enhanced by exposure to radiation. An increased risk of lymphoma, soft-tissue sarcoma and bladder cancer seemed due to radiation therapy, whereas excesses for cancers of the breast, uterine corpus, and large bowel appear to reflect an underlying host predisposition shared with ovarian cancer. A system for monitoring the risk of second primaries is now being developed in collaboration with the NCI Division of Cancer Treatment, utilizing the protocols of several clinical trial groups.

Immunosuppressive agents--The Branch updated an analysis of data from the Renal Transplant Registry of the American College of Surgeons, now covering over 16,000 recipients treated with immunosuppressive agents (28). The risk of histiocytic lymphoma was approximately 150 times that expected from the experience of the general population (29). This risk had two unusual characteristics: an explosive onset within the first year of transplantation and a predilection for brain involvement. There was no appreciable variation in risk of lymphoma by the type of renal disease, relationship to the donor, or single versus multiple transplants. The risk of other cancers was elevated two-fold, but the excess did not appear until three years after transplantation and became progressively larger with increasing follow-up. The excess risk did not affect all tumors across-the-board,as might be anticipated by the immunosurveillance hypothesis, but was limited to particular neoplasms, including soft tissue sarcoma, adenocarcinoma of the lung, tumors of the liver and bile duct, cancers of the thyroid and lower urinary tract, melanoma, and perhaps cervical cancer. Since the renal registry of the American College of Surgeons was recently disbanded, continued follow-up will utilize the registry of patients with end-stage renal disease (transplant and dialysis patients) now being maintained by the HEW Bureau of Quality Control. In addition, studies are being developed to evaluate whether immunosuppressive drugs increase the risk of cancer in various conditions (e.g., collagen disorders) other than transplantation.

Occupation

During this year the Branch developed a program in occupational studies, an area that in the past has provided much of the information known about chemical carcinogenesis in man (30). Emphasis is given to research designed to explain the "hot spots" identified by the county-by-county survey of cancer mortality, to evaluate the effects of industrial exposures that cross plant boundaries into the general environment, and to provide insights into the basic mechanisms of carcinogenesis. Most of the effort this year has gone into the initiation of specific projects, including the selection of occupational groups, identification and evaluation of existing data sources, and securing cooperation of appropriate parties. Contacts were made with industrial firms, labor unions, professional organizations, state agencies, and the Social Security Administration. Underway are proportionate mortality and follow-up studies of workers in dry cleaning shops, oil refining and petrochemical plants, chromate paint application, leather industry, pharmaceutical and biological manufacturing, and a large manufacturing plant employing machinists, foundry workers, welders, and platers. In addition,

a few exploratory studies were reported using the method of proportionate mortality. Construction machinery operators had an increased mortality from lung cancer, particularly at older ages, that may be related to engine exhaust fumes that contain hydrocarbons (31). A 3-fold excess of fatal accidents outside the home indicated hazardous conditions in the work environment. Stationary engineers and firemen had a 2-fold excess of oral and rectal cancers, and a 20% increase in lung cancer, which may be caused by exposures to hydrazine compounds, chromates, or possibly asbestos (32). An excess mortality from heart disease was found, possibly related to carbon monoxide exposure. Another survey involved printing pressmen exposed to oil mist, solvents, dyes and pigments that may be carcinogenic (33). At young ages oral cancer was excessive among newspaper pressmen and pancreatic cancer among commercial pressmen, and a slight increase was seen for lung cancer in both groups. Mortality was elevated also for coronary heart disease, perhaps due to carbon disulfide exposure. The effect of printing exposures on cancer risk has been suggested also by reports of occupational case clusters and by detection of high-risk communities where printing ink is manufactured (34). To evaluate the cancer risk associated with the leather industry, study was made of medical records at the Roswell Park Memorial Institute in Buffalo (35). Bladder cancer occurred excessively among men and women employed in plants manufacturing leather and leather products, and may be related to exposures to azo and other synthetic dyes. An increased risk was seen also for cancers of the oral cavity and larynx, and for malignant lymphomas. The association with oral cancer is consistent with the elevated rates of this tumor in leather-industry communities (15).

Radiation

Ionizing--Using resources of the Radiation Effects Research Foundation (formerly Atomic Bomb Casualty Commission), one Branch member completed studies that add new cancers (lymphomas and cancers of esophagus, stomach, and urinary tract) to the list of those known to be caused by ionizing radiation from the 1945 A-bomb explosions. The influence of age at exposure was strong but varied according to site. Solid tumors as a group now outweigh leukemia as a radiation effect, in terms of absolute risk. Since the effect appears generally stronger in Hiroshima than Nagasaki, it would seem that neutrons are more potent than gamma rays as carcinogens. Studies of breast cancer revealed a dose-response relationship that was approximately linear at low doses with an effect evident at doses as low as 17 rads (36,37). The risk was greatest among women exposed at younger ages, but latency seemed unrelated to dose. The latency for lung cancer also was unaffected by dose, whereas leukemia tended to occur earlier in the heavily exposed groups (38). The mechanisms for leukemogenesis may be fundamentally different from carcinogenesis generally, since age-related events appear to be required before radiogenic tumors of the breast and lung can be induced. Studies are planned to evaluate the interaction between radiation and susceptibility factors in the development of breast cancer.

A number of other projects were initiated this year, including a follow-up study to evaluate the risk of thyroid tumors among children exposed

to 131-iodine thyroid uptake tests, in collaboration with the Bureau of Radiological Health (Food and Drug Administration). Members of the Branch also helped prepare committee reports to assess the risks and benefits of mammography screening for breast cancer (NCI), to review and reanalyze a mortality survey of cancer among plutonium workers (Energy Research and Development Agency), and to reevaluate the biological effects of ionizing radiation (National Academy of Sciences).

Ultraviolet--In collaboration with the Biometry Branch, a study was initiated to clarify the role of solar UV-radiation in the development of non-melanoma skin cancer, by means of a demographic survey using tumor registries around the country and a case-control study to clarify the influence of various host and environmental cofactors. A north-south gradient for skin melanoma confirmed the influence of sunlight on the risk of this tumor, while no such effect was seen for ocular melanoma (39). However, both skin and ocular melanomas were more common in whites than blacks, and predominated in females at younger ages and males after middle life.

Family Studies

Epidemiologic patterns of cancer have suggested that the environment contributes much more to cancer risk than do genetic factors. Perhaps in the general run of patients, inherited susceptibility and interactions with environmental influences are too subtle or complex for detection by ordinary epidemiologic means. On occasion, when susceptibility is conspicuous, as in cancer-prone families, research opportunities are provided that may clarify the role of genetic mechanisms and environmental interactions in carcinogenesis. Studies on high-risk families are conducted along with the Clinical Epidemiology Branch, and with clinical and laboratory scientists at NIH and other institutions. The clinical patterns of familial cancer and the interdisciplinary research approach were reviewed this year (40-42).

Studies of several cancer-prone families were reported this year, and whenever possible new laboratory developments were utilized. For example, in three of four sibs affected with chronic lymphocytic leukemia, the peripheral blood leukemic cells shared δ-heavy and κ-light chains as the only detectable surface immunoglobulins, suggesting an inherited defect in a class of lymphocytes identified at a biomolecular level (43). All members of the family worked in a dry cleaning shop and had heavy exposure to chemical solvents, particularly perchloroethylene (44). Genetic-environmental interaction was suggested also by a 22-year-old woman who developed breast cancer 15 years after radiotherapy to the lung for metastatic Wilm's tumor (45). Her 32-year-old mother died of bilateral breast cancer, suggesting familial predisposition to radiogenic cancer. In another family, pancreatic cancer arose in a father and son, both exposed heavily to occupational chemicals and probably to aflatoxin (46). A new syndrome was suggested by a brother and sister who succumbed to glioblastoma associated with hepatic focal nodular hyperplasia and various aberrant growths, suggesting a hamartomatous malformation syndrome predisposing to brain tumors (47). The possibility of transplacental carcinogenesis was suggested by the occurrence of malignant mesenchymoma in a young man with hydantoin-

associated anomalies (48). In addition, study was made of seven families prone to malignant melanoma, and all had a syndrome of atypical melanocytic lesions. Using this mole pattern as a clinical marker of susceptibility, six individuals were found to have melanoma through careful skin examinations.

Immunoepidemiology

In addition to the evaluation of immunosuppressive drugs and immune defects in high-risk families, described above, various projects were undertaken to clarify the role of immunologic defects in carcinogenesis. In collaboration with the University of Minnesota, a registry of cancers occurring in patients with primary immunodeficiency syndromes has been maintained (49). This year a concerted effort was made to add new cases to the registry. Lymphomas continued to predominate but, unlike the transplant series, there was an excess risk of Hodgkin's disease, lymphocytic leukemia and brain tumors in children, and stomach cancer in adults.

Various disorders with "secondary" immunologic defects have been associated with an excess risk of cancer, primarily lymphomas. However, the immunodeficiency is varied and complex, often associated with immunostimulation and lymphoid hyper-reactivity, and related to cancer in a manner that seems less precise than with the primary immunodeficiency syndromes. A follow-up study of patients with Sjogren's syndrome, an autoimmune disease, revealed a 100-fold excess risk of non-Hodgkin's lymphoma compared to the general population. Six cases of Waldenstrom's macroglobulinemia occurred, clearly in excess of expectation, although no population rates for this condition exist. The risk of lymphoma seemed enhanced by parotid enlargement or possibly the low-dose radiation given to reduce this swelling. In another survey, involving patients with chronic lymphocytic leukemia reported to the End Results Program, preliminary analysis suggests an excess risk of second primary tumors, particularly melanoma, lung cancer, soft-tissue sarcoma, and possibly rectal cancer (49).

In collaboration with the Medical Follow-Up Agency (National Academy of Sciences) other groups are under survey. A follow-up of 740 servicemen splenectomized because of trauma during World War II revealed a significant excess of pneumonia and ischemic heart disease, and a suggestive excess of liver cirrhosis (50). The findings confirm the increased risk of fatal infections previously associated with asplenia, but there was no predisposition to cancer. This may be related to the sparing of cell-mediated immunity, which is impaired in the various immunodeficiency states predisposing to cancer. Postsplenectomy thrombocytosis and hypercoagulability may account for the increased risk of fatal myocardial ischemia in this group, demonstrating that studies designed to understand one disease often provide data that may shed light on another.

The immunosurveillance concept indicates not only that weakening of the immune system leads to cancer, but that its strengthening may be protective. A few reports have suggested that the risk of cancer is lower than usual among allergic people, but the preliminary results of a follow-up of

servicemen with asthma point to an excess of cancer, especially of the lung and pancreas. Also underway is a study of cancer risk in servicemen with sarcoidosis, which is associated with impaired cellular immunity and lymphoproliferation. Planning was completed with the Veterans Administration for studies relating several immunological disorders to cancer risk, but this project has been delayed due to legal difficulties created by certain provisions of the Privacy Act. Nearing completion is a follow-up study to evaluate the fate of 3,000 former employees of Fort Detrick, Maryland, who were "hyperimmunized" by repeated vaccination.

A review of the epidemiologic evidence compiled to date revealed little support for the immunosurveillance theory of cancer, except in the case of lymphoid tumors (49). Some observations suggest that failing immunosurveillance may predispose to skin cancer, and to adenocarcinomas of the lung and stomach, yet these tumors are not linked to the different immunodeficiency states as pervasively as lymphoid tumors.

Viruses

Viruses have not been causally tied to the origins of any human cancer, but some relationships are under active investigation. Using an interagency agreement with the U.S. Navy, a study in Taiwan is being conducted to evaluate the risk of hepatoma in chronic carriers of hepatitis B infection. In conjunction with Case-Western University, a follow-up survey of newborn children who received polio vaccine containing SV40, an oncogenic virus to laboratory animals, has revealed no unusual patterns of disease after 15 years. Hodgkin's disease in young people is reported by some investigators to spread by interpersonal contact, so with the Followup Agency a study is being designed to evaluate the patterns of contact among servicemen who develop this tumor.

In collaboration with investigators from Ankara, and the NCI Viral Oncology Program, study was made of the high frequency of Turkish children with acute myelogenous leukemia, often presenting with chloroma-like deposits in the eye and orbit (51). Laboratory studies uncovered no abnormalities of etiologic significance, but a group-specific antigen (type C virus?) was identified in one patient by radio-immunoassay of orbital tumor extract. Two cases of Burkitt's lymphoma, which may be virus-induced,were reported with severe hypercalcemia, a previously unrecognized complication of this tumor (52).

In association with Meloy Laboratories, studies were conducted to evaluate the utility of the SV40 transformation test as an indicator of cancer proneness (53-56). The T-antigen expression in SV40-infected skin fibroblasts was elevated in several cytogenetic syndromes and in two families prone to acute myelogenous leukemia.

Epizoology

In 1964 the National Cancer Institute initiated the Veterinary Medical Data Program, a resource shared with the Clinical Epidemiology Branch. By epidemiologic comparisons with human cancer, studies in domestic animals are designed to clarify etiologic factors in human cancer, uncover animal

models (e.g., breeds) that may be useful in further research, and identify sentinels that may act as early predictors of environmental hazards to man. This year a study of renal carcinoma in dogs revealed a male predominance and age distribution similar to the patterns in man, suggesting that endogenous sex hormones may be involved in susceptibility to this tumor (57). Ovarian tumors in dogs showed histologic and epidemiologic similarities to man, plus an excess risk of epithelial tumors in the Pointer breed and granulosa-theca cell tumors in the English Bulldog, and a sparing of mongrel dogs from malignant but not benign tumors (58). Testicular tumors in dogs were equally represented by three cell types (seminoma, Sertoli cell tumor and interstitial cell tumor) and several pure breeds were prone to different tumors (59). Cryptorchid dogs had a 14-fold excess risk of testicular tumor while male dogs with inguinal hernia had a 5-fold excess; the predisposition of the Shetland Sheepdog suggests an appropriate model to study mechanisms for testicular maldescent and tumorigenesis. In addition, perianal gland tumors in dogs showed a 6-fold increase among males, consistent with evidence for androgen dependency, and the excess risk among both sexes of Cocker Spaniels suggests a model for studies of hormone-related tumors in human beings (60). Another androgen-related disorder in dogs seems to be perineal hernia, showing evidence for a therapeutic effect from orchiectomy together with herniorrhaphy (61). By contrast, canine bladder cancer was much more common in females, possibly a result of less frequent urination, so that surveillance of this tumor may provide an early warning of emerging environmental hazards for man (62). Finally, a review was made of arsenical poisoning in domestic animals, with acute toxicity from herbicides or insecticides being the most common effect, particularly in dogs and cattle (63).

Methodologic Studies

The Branch was involved this year in the development and evaluation of statistical methods for use in epidemiologic studies. A new approach to odds-ratio estimation in matched-pair studies was devised (64) and rules for choosing sample sizes for cases and controls to provide the most precise estimates of relative risk were developed (65,66). The effect of limited observational periods on evaluating the possibility of an infectious nature to Hodgkin's disease was considered (67). One member conducted a seminar series on the principles of epidemiologic research attended by scientists throughout NIH. In addition, a set of computer programs to analyze a variety of epidemiologic problems were written for use on the Hewlett-Packard 67 calculators located in the Branch.

Reviews

The Branch often provides comprehensive yet critical reviews of etiologic factors in cancer. This year surveys were published on lung cancer (68,69), pancreatic cancer (70), and multiple primary neoplasms (71). The epidemiologic evidence for immunologic (49) and genetic (72) factors in cancer etiology was presented, and the relative contributions of host and environmental determinants were examined (73,74). The relation between teratogenesis and carcinogenesis was illustrated by a review of congenital hemihypertrophy, a malformation that increases the risk of embryonal neoplasms involving the kidney, adrenal cortex, and liver (75).

In association with the Clinical Epidemiology Branch, a conference was held in late 1975 to assess the role of genetics in human cancer. The proceedings was published this year (76). In 46 chapters the book, Genetics of Human Cancer, covers recent advances in population genetics, malformation-cancer syndromes, cancer cytogenetics, monogenic syndromes, familial aggregation of cancer, genetic markers, cellular biology, general theories of pathogenesis, and opportunities for future research.

As a sequel to the 1975 monograph, Persons at High Risk of Cancer, one Branch member participated in a UICC international workshop entitled Cancer Risks by Site (77). The purpose was to review and classify information on high-risk groups according to cancer site in a manner that would enhance further efforts in cancer control and research.

OTHER ACTIVITIES

The Branch provides a liaison for epidemiologic research in the National Cancer Program and for environmental cancer studies being conducted in various agencies in the federal government. The staff contributes advice and support to clinicians, experimentalists, and public health officials. Members serve on the editorial boards of various journals and on several advisory groups and committees connected with cancer centers, federal agencies, and other national and international activities. This year the Branch helped to organize and chair several conferences, and one member is planning a workshop on biostatistical methods in cancer research under the aegis of the U.S.-Japan Cooperative Cancer Research Program.

PLANS

The allocation this year of new positions to the Branch has permitted an expansion of epidemiologic activity at the National Cancer Institute, and has enabled the Branch to serve more effectively as a focus for initiatives in environmental cancer studies. In the further development of the research program, the Branch plans to expand its collaborative studies with other federal and state agencies having valuable statistical or environmental data, and with component units of the Surveillance, Epidemiology and End Results (SEER) Program that has population-based cancer registries located around the country. By enlarging the support services contract, the Branch plans to intensify efforts to identify the environmental causes of cancer in high-risk communities previously identified by the mapping of cancer mortality on a county level. Many promising leads are being pursued, but more studies are needed to extend the coverage of high-risk areas in various parts of the country. Continued emphasis will be given to studies to clarify the carcinogenic potential of several drugs, particularly hormones and cytotoxic agents. A major new program will utilize the several NCI-sponsored cancer therapy trials to monitor the occurrence of new cancers that might result from treatment. Investigations will be stepped up to assess the carcinogenic influence of chemical pollutants in the workplace and in the general environment, including water and air. Occupational studies will emphasize hazards that may spread beyond plant confines or contribute to the "hot spots" on the cancer maps. Epidemiologic and multi-

disciplinary studies will be used to evaluate the nutritional constituents and mechanisms involved in cancer etiology, and further collaboration with laboratory investigators will help clarify the role of candidate viruses and genetic susceptibility in carcinogenesis. Thus, the enlarged program is enabling the Branch to augment existing project areas and initiate new lines of research. The developments this year should lead to a comprehensive, flexible, and balanced program with a greater capacity to generate leads and help settle key questions in cancer etiology.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04378-03-EEB

PERIOD COVERED

July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

U.S. Cancer Mortality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.J. Mason	Health Statistician	EEB NCI
OTHER:	J.F. Fraumeni	Chief, Environmental Epidemiology Branch	EEB NCI
	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
	W.J. Blot	Health Statistician	EEB NCI
	B.L. Stephenson	Computer Systems Specialist	EEB NCI
	R.I. Ramsbottom	Computer Systems Specialist	EEB NCI

COOPERATING UNITS (if any)

National Center for Health Statistics, Bureau of the Census

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study. Publications from this area of interest have facilitated the design of ongoing analytical investigations to test specific etiologic hypotheses. Included among these are studies of lung cancer in coastal Georgia, bladder cancer in New Jersey, colon cancer in rural Nebraska, and liver cancer in Southeast Texas. An analysis of cancer mortality among nonwhites revealed striking similarities in geographic patterns for cancers of the breast, colon, rectum, and esophagus. Site-specific analyses for cancers of the esophagus, buccal cavity and pharynx, and large bowel revealed associations with ethnicity and industry. Industry-specific studies revealed elevated nasal cancer rates in counties with large numbers employed in the furniture industry, and elevated rates for cancers of the lung, nasal cavity and sinuses, and skin in counties where the petroleum industry is most heavily concentrated. We are continuing to expand our data sets, both of reported deaths and measures of exposure.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study.

Methods Employed:

Computer analysis of over 6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated year by year, and expanding. Base populations for Whites, Blacks, and all nonwhites are maintained for the entire time period. The system for the total U.S. provides race, sex, ICD, and age-specific analyses of cancer mortality consisting of numbers dead, populations at risk, age-specific and age-adjusted rates, a measure of the variability in the age-adjusted rate, and a linear regression of the age-adjusted rates as a function of time. This system is fully operational.

A system for the analysis of cancer mortality for individual counties with the flexibilities of the national system is currently operational.

Major Findings:

A systematic analysis of cancer mortality among U.S. nonwhites revealed marked similarities between Whites and nonwhites for cancers of the breast, colon, rectum and esophagus, which showed generally high rates in the North and low rates in the South. Cancers of the larynx, bladder and ovary also had above-average rates in the North. For lung cancer, both White and nonwhite males experienced high rates in northern areas; however, the pattern of high lung cancer rates for White males along the Gulf and Southeast Atlantic coasts was much less pronounced among nonwhites. Esophageal cancer rates among White and nonwhite males were high in the urban North, but only nonwhites showed an excess in southern coastal areas. For both Whites and nonwhites, cancers of the uterine cervix had above-average rates primarily in rural areas of the South. Rates were also calculated for Blacks, American Indians, Chinese and Japanese. Compared to other racial groups, Blacks experienced high rates for cancers of the mouth and throat, esophagus, stomach, pancreas, larynx, lung, bladder, cervix, and multiple myeloma. American Indians experienced more cancers of the gallbladder, bile ducts and liver; Chinese had high rates for cancers of the nasopharynx and Japanese had increased stomach cancer rates.

A recent report by the National Health Federation, a private agency, related cancer mortality patterns in the United States to fluoridation of water supplies, triggering much public health concern and some political response. To clarify the issues raised, we studied cancer mortality and incidence statistics for U.S. counties, 1950-69. No trends could be ascribed to the consumption of water that is artifically or naturally fluoridated.

A survey of esophageal cancer mortality among U.S. counties, 1950-69, revealed higher rates among males in the Northeast and among females in the South. Throughout the country mortality among White males and nonwhites of both sexes increased markedly with urbanization. A nearly 2:1 excess among nonwhites was observed. Clustering of exceptionally high rates among nonwhites was observed along the Southeast Atlantic coast, particularly around Charleston, South Carolina. Among Whites, inverse associations with socioeconomic indices and positive associations with ethnicity were detected. Slightly higher rates among females were seen in southern counties with textile or paper industries.

Mortality rates for large bowel cancer were consistently elevated in counties with large populations, higher income and education levels, and high percentages of residents of Irish, German, or Czechoslovakian descent. The urban, socioeconomic, and ethnic factors were each linked to large bowel cancer, but they only explained part of the predominance of this tumor in the Northern United States. A survey was made of the limited data available on dietary habits by region and on alcohol sales by county. The concomitant variation with mortality rates for large bowel cancer was not impressive.

A survey of buccal cavity and pharynx cancer mortality in United States counties, 1950-69, revealed elevated rates among males in urban areas throughout the country and among females in the rural South. Indirect evidence suggests that the urban gradient in males results largely from alcoholic beverage consumption and tobacco smoking, while the Southern excess in females is related primarily to oral snuff use. Mortality was elevated also in counties with leather, paper, and chemical manufacturing industries (in males), and apparel and textile industries (in females). The industrial correlations in both sexes remained after control for urbanization and other demographic factors, but snuff use by Southern women in the textile and apparel industries may have influenced this association for females.

U.S. counties were identified in which a substantial proportion of the population was employed in the furniture industry. A comparison was made of the cancer-mortality rates for White males in these counties with the cancer rates in a group of counties of similar demographic characteristics but with no involvement in the industry. Statistically significant excesses were seen for nasal cancer, melanoma, and multiple myeloma. Within each of the four region-population subdivisions, the rates for these three cancers were greater in the furniture industry counties than in the control counties.

A survey of cancer mortality, 1950-69, was conducted in U.S. counties where the petroleum industry is most heavily concentrated. Male residents experienced significantly higher rates for cancers of the lung, the nasal cavity and sinuses, and the skin (including malignant melanoma) when compared to counties with similar demographic characteristics. Further study is needed to determine if these patterns result from exposure to chemical carcinogens, including polycyclic hydrocarbons, involved in the manufacturing of petroleum.

A survey of cancer incidence among Alaska Natives for the 5-year period 1969-73 revealed fewer cancer cases overall than expected in relation to U.S. rates, but significantly increased risk for certain cancer sites: the nasopharynx in both sexes (with excesses over 15-fold), the liver in males, and the salivary glands, gallbladder, kidney and thyroid in females. Compared with earlier reports, the observations suggest marked changes in cancer incidence among Alaska Natives over the past two decades, with declines in esophageal and invasive cervical cancers, and increases in cancers of the lung, colon and rectum.

U.S. mortality and incidence statistics for nasopharyngeal cancer showed a 4-fold excess risk of sarcomas in White children under age 10, and a 4-fold to 7-fold excess of carcinomas in teen-age blacks. Mortality from nasopharyngealcarcinomas in young people was greater in the South than in the North, with the excess mortality in Blacks linked to rural residence and low socioeconomic status. These and other characteristics of nasopharyngeal carcinoma in young persons suggested that environmental (perhaps infectious) agents are involved in this age group. These patterns contrasted with nasopharyngeal carcinomas developing after age 25, when the rates predominated in Chinese Americans. Nasopharyngeal cancer in the United States had three age peaks, with racial and epidemiologic distinctions that seemed to reflect different etiologies.

Publications

Mason, T.J., McKay, F.W., Hoover, R., Blot, W.J., Fraumeni, J.F., Jr.: Atlas of Cancer Mortality Among U.S. Nonwhites: 1950-1969, DHEW Publication No. (NIH) 76-1204, 1976, 142 pp.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04401-03-EEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunologic Factors in Cancer Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
OTHER:	J.F. Fraumeni	Chief, Environmental Epidemiology Branch	EEB NCI
	T.J. Mason	Statistician Health	EEB NCI

COOPERATING UNITS (if any)
University of Minnesota, Immunodeficiency Cancer Registry
Veterans Administration, Biometrics Division
Veterans Follow-up Agency of the National Academy of Sciences

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	0.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study populations with altered immunologic states experiencing unusual rates of malignancy. Risks of cancer of different sites are quantified for various groups of such patients, compared with each other, and characteristics and possible determinants of unusual risks are sought. The populations studies include renal transplant recipients, patients with inherited and acquired immune deficiency syndromes, groups of immune stimulated persons (e.g. hyperimmunized, B.C.G. vaccination) and patients experiencing conditions for which immune sequelae are suspect but unknown (e.g. splenectomy patients). Both cohort studies of these groups of patients and case-control studies of such patients who also developed cancer are conducted.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To identify and study populations with altered immunologic states experiencing unusual rates of malignancy. (2) To study these populations in order to identify the characteristics and possible determinants of any unusual risk.

Studies and Methods Employed: (1) An ongoing follow-up study of the malignancy experiences of renal transplant recipients was continued and updated. The malignancy experience of over 16,000 patients who received renal transplants was evaluated by comparing the numbers observed during the follow-up of these patients with that expected based on cancer incidence rates prevailing in the general population.

(2) Tissue typing information from both the transplant recipient and the donor have been obtained for a large number of the patients being followed. Analysis is currently underway of the differences in HL-A type of the transplant recipients who subsequently develop a malignancy versus the corresponding information among those transplant recipients who did not develop a malignancy.

(3) In collaboration with the University of Minnesota, through the contract mechanism (NO1-CP-43384), a registry of malignancy in patients with immune deficiency syndromes was maintained. In the past year, a large number of previously unreported cases have been added to the registry through a concerted effort to contact previously noncontributing immunologists and by a survey of those who have already reported to the registry. In addition, a subgroup of clinics which see relatively large numbers of these patients and which are willing to cooperate at a more intensified level was identified. An attempt will be made to monitor these clinics in order to generate rates of malignancy among well-characterized groups of patients with specific immune deficiency syndromes.

(4) A follow-up study is underway of the malignancy, mortality, and reproductive experience of 3,000 former employees of Ft. Detrick (formerly a biologic warfare research center) in Frederick, Maryland. A large proportion of these workers fall into a group which would be considered "hyperimmunized". The personnel and immunization records for this population have been abstracted and computerized. An initial mailing has been sent in order to identify those former employees who have died and the health status of those who are still alive. Efforts are currently underway to locate those whose questionnaire was not deliverable and to gain the cooperation of those who received the questionnaire but as yet have not responded.

(5) Several reviews of the status of the relationship between malignancy and immunology have been conducted.

(6) Planning was completed for a series of studies in collaboration with the Veterans Administration. In these studies, the malignancy experiences of a number of groups of patients with acquired immune deficiency states and

others undergoing procedures or drug therapy involving immunosuppression will be compared with that of the general population. The project has been delayed due to legal difficulties created by certain provisions of the Privacy Act.

(7) A follow-up study of the malignancy and mortality experiences of a group of patients with Sjogren's syndrome has been conducted. This is an autoimmune disease associated with both immunostimulation and a minor degree of immune suppression. The experience of this group has been compared to that of the general population.

(8) A follow-up of a large number of patients with chronic lymphocytic leukemia was done to assess their risk of second tumors of other types. Again, the experience of this group was compared to the cancer incidence of the general population of the same age, race and sex.

(9) A follow-up study of 740 men who had their spleen removed during World War II because of trauma was conducted to assess the influence of splenectomy on subsequent risk of malignancy and subsequent mortality experiences. The numbers of deaths due to cancer and other causes were compared to the number expected based on rates of the general population. In addition the observed numbers were compared to those expected based on a follow-up of 740 men, matched to the splenectomy patients on age and year of admission to hospital.

Major Findings: (1) The risk of reticulum cell sarcoma among renal transplant recipients is approximately 150 times that expected based on the experience of the general population. This risk of lymphoma has two very unusual characteristics: it occurs with an explosive onset, achieving this high level of relative risk within the year following transplantation; it has an extremely high predilection for brain involvement. There is no evidence of any appreciable variation in risk of lymphoma by the type of renal disease of the recipient, the relationship of the donor, or single versus multiple transplants. There is a possibility that the relative risk of lymphoma is greater among children than among adults.

(2) Among renal transplant recipients cancers other than lymphoma occur at approximately twice the expected rate. This excess does not appear until two years following transplantation, becomes progressively larger with increasing follow-up, and is due largely to excesses of soft-tissue sarcomas, adenocarcinomas of the lung, primary tumors of the liver and bile ducts, cancers of the lower urinary tract, thyroid cancers, and perhaps cancers of the uterine cervix. No excesses were noted for many sites, including a number of the more common ones (stomach, colon, rectum, and breast).

(3) Persons with immune-deficiency syndromes have a high risk of lymphoma, similar to that among transplant recipients. Unlike the transplant population, however, they also appear to be at high risk of Hodgkin's disease, lymphatic leukemia (children), and stomach cancer (adults). A

previously reported excess of malignancy among individuals with x-linked (Bruton's) agammaglobulinemia does not appear in the current data.

(4) Patients with Sjogren's syndrome have approximately 100 times the risk of non-Hodgkin's lymphoma of that in the general population. The relative risk for lymphoma in patients with sicca complex alone resembles that for Sjogren's patients with rheumatoid arthritis. Six cases of Waldenstrom's macroglobulinemia occurred among the patients in this study, clearly vastly in excess of that expected, although no rates for this condition exist. Other non-skin tumors occurred at their expected frequency. There was some evidence that either parotid enlargement or low dose therapeutic irradiation to reduce parotid enlargement may have predisposed lymphoma malignancy in Sjogren's syndrome.

(5) Men having undergone a splenectomy as a result of trauma have mortality rates from malignancy similar to those experienced by the general population. There were excess deaths among the splenectomy patients for pneumonia,ischemic heart disease and cirrhosis.

Proposed Course of Project: (1) The transplant registry of the American College of Surgeons has been discontinued. Currently there is a registry of patients with end-stage renal disease that is being maintained by the Bureau of Quality Control of H.E.W. In this year, attempts will be made to assess the usefulness of this data base for continuing and expanding these studies of malignancy in patients receiving immunosuppressive drugs. Hopefully, this resource will also allow the assessment of the risk of malignancy among patients with chronic uremia, an immunosuppressive condition itself. (2) The collaboration with the immunodeficiency and cancer registry will continue with periodic evaluation of the data collected. (3) A case-control study of the differences in tissue typing information for those transplant recipients going on to develop malignancy and those not progressing to malignancy will be conducted. (4) Continued efforts will be made to follow up the hyperimmunized group from Ft. Detrick, and a mortality analysis of this group will be conducted. (5) Attempts will be made to expand the study of Sjogren's syndrome and other diseases characterized by altered immunologic states by contacting centers seeing large groups of such patients to elicit their cooperation in a collaborative study. (6) If all legal problems can be resolved, the series of studies with the Veterans Administration should be initiated.

Publications

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04410-01-EEB

PERIOD COVERED

July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Cancer-Prone Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Br.	EEB	NCI
OTHER:	W.A. Blattner	Clinical Investigator	EEB	NCI
	R.R. Reimer	Clinical Associate	EEB	NCI
	M.H. Greene	Staff Associate	EEB	NCI

COOPERATING UNITS (if any)

Clinical Epidemiology Branch, NCI

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct and coordinate interdisciplinary studies on cancer-prone families in order to clarify the role of genetic mechanisms and host-environmental interactions in carcinogenesis. Clinical studies of high-risk families have suggested etiologic relationships between cancers, constellations of which may cluster in individuals (multiple primary neoplasms) or may be scattered over the family tree (familial neoplastic syndromes). Discovery of laboratory abnormalities in certain cancer-prone families, such as subclinical immunodeficiency in family members predisposed to lymphoma, or premalignant conditions in individuals prone to colon cancer and melanoma, have provided insights into the mechanism of susceptibility to cancer.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To document the occurrence of cancer in high-risk families, and to study such families by clinical, epidemiologic, and laboratory investigations, in an effort to elucidate genetic mechanisms and host-environmental interactions contributing to carcinogenesis. To coordinate the distribution of tissue and blood specimens from such families to interested investigators for etiologic study by cytogenetic, immunologic, viral, endocrine, biochemical, tissue culture, and other methods.

Methods Employed: Interview of cancer patients with respect to prior medical and environmental history and family occurrence of cancer and other disorders; documentation of history by appropriate vital records, hospital charts, and review of pathology specimens; collection of biological specimens from members of high-risk families, and circulation to appropriate laboratories for etiologic study.

Major Findings: During the past year a monograph, Genetics of Human Cancer, Raven Press, New York, 1977. was published under the editorship of Dr. John J. Mulvihill and Dr. Robert W. Miller of the Clinical Epidemiology Branch, and Dr. Joseph F. Fraumeni Jr., Chief, Environmental Epidemiology Branch. This comprehensive volume reviews diverse information concerning the role of host genetic factors in the etiology of cancer. Chapters by branch members include "Clinical Pattern of Family Cancer" and "Family Studies: The Interdisciplinary Approach."

A non-polyposis, colon cancer-prone family was evaluated, and cytogenetic and immunologic abnormalities which may contribute to the cancer risk in this family were reported.

Predisposition to radiogenic cancer was observed in a high risk individual with Wilms' tumor who developed a second primary, breast cancer in the site of prior radiation therapy. The importance of long-term follow-up for adverse effects in such patients was emphasized. A rare familial association that may be related to an unusual exposure to aflatoxin and chemicals was noted, and the need for careful environmental histories in familial aggregation was stressed. The relation of teratogens to carcinogens was suggested in an individual born with Dilantin-associated congenital anomalies who subsequently developed an embryonic tumor, mesenchymoma. In this case the family history of seizures provided clues for a possible transplacental carcinogenic effect for this drug. Adolescent siblings developed acute lymphoblastic leukemia within three weeks of each other. Immunogenetic studies suggest a recessively inherited susceptibility factor similar to that reported in certain animal models.

Several families with an unusual pattern of cutaneous moles and susceptibility to malignant melanoma have provided new insights into the pathogenesis of this malignancy. The clinical evaluation of these lesions has provided clues about the biology of malignant transformation. Laboratory investigation has revealed immunologic correlates to pathologically observed immunocyte response to the tumor, and may help explain why certain of these families are more resistant

to malignant evolution than others. Continued collaboration with Dr. Martin Lipkin at Memorial Sloan Kettering Institute, has provided an opportunity for evaluationg the in vitro correlates of premalignant changes in colon cancer families.

The histopathologic correlates of malignant lymphoma have been reviewed on a large number of families prone to this disease. Although family-specific patterns were observed, as a group the trend was toward less common types, and types that share a common immunologic cell type. Most striking was the recurrent observation that clinical and subclinical immune deficiency seemed to play an important role. The observation that environmental antigenic factors may have predisposed to malignant lymphoma in an unusual site (Waldeyer's Ring) in a family was supported by the observation that immune abnormalities were found in both family members and their spouses, who were sharing the same environment, but not in those away from this exposure.

An analysis on 30 families prone to cancer who were tested using the SV 40 virus T-antigen test, indicates that a single gene cannot account for the regulation of viral material. This polygenic hypothesis points to the need for careful evaluation of the significance of an abnormal result as it relates to cancer susceptibility.

Significance to Biomedical Research and the Program of the Institute: Studies of cancer-prone families may help to detect genetic mechanisms and host-environment interactions in carcinogenesis, and also help to identify those individuals most likely to benefit from screening programs aimed at early diagnosis of cancer.

Proposed Course of Project: The same approach will be continued. New laboratory techniques will be introduced into the project as necessary.

Publications

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Law, I.P., Hollinshead, A.C., Whang-Peng, J., Dean, J.H., Oldham, R.K., Herberman R.B., and Rhode, M.C. Familial Occurrence of Colon and Uterine Carcinoma and of Lymphoproliferative Malignancies: II. Chromosomal and Immunologic Abnormalities. Cancer. 3:1229-1236, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04411-02-EEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Cancer and Related Conditions in Domestic Animals: Epidemiologic Comparisons with Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard M. Hayes, Jr.	Staff Associate	EEB NCI
OTHER:	Joseph F. Fraumeni, Jr.	Chief, Environmental Epidemiology	EEB NCI
	Robert Hoover	Head, Env. Studies Section	EEB NCI

COOPERATING UNITS (if any)

Clinical Epidemiology Branch, NCI

LAB/BRANCH
Environmental Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NCI,NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The continuing purpose of this project is to identify animal models applicable to further research into the etiology of cancer in humans. Additionally, as cases accumulate, the probability increases that domestic pets with some types of spontaneous cancers can be identified as representing the effects of low level environmental exposure to carcinogenic agents. The frequency of cancer in these animals would serve as a warning of general environmental hazards to man. The topics of current interest include: 1) environmentally influenced cancer in dogs relative to the level of industrialization in their county of residence; 2) a case-control study of 1500 cases of canine mammary neoplasms to determine risk associated with age, breed, obesity, prior ovariohysterectomy and prior benign mammary neoplasia; 3) the epidemiologic features of gastro-intestinal cancers, neoplasms of dental tissue and prostatic cancer in dogs; 4) a case-control study of the long term effects of Promone in dogs; 5) a morbidity study about dogs exposed to kepone.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To investigate the distribution of cancer and related conditions in domestic animals in order to 1) clarify etiologic factors in humans, 2) identify animal models useful in research, and 3) identify sentinels which may act as early predictors of environmental hazards to man.

Methods Employed: Animals with the disease under investigation are identified when possible, from the medical abstract records sent to NCI's Veterinary Medical Data Program. A comparison population-at-risk is constructed from all patients seen by participants during the same time period under consideration. The diseased animals are characterized by relative risk techniques for various factors (i.e., age, breed, sex). Other analytical techniques employed may include case-control comparison for other factors associated with the disease in man. Other animals under study are identified, wherever the resource is available.

Major Findings: A retrospective study of renal carcinoma in dogs shows inheritance is not a risk factor. However, a male predominance was evident and resembled the age pattern of risk seen in man.

Ovarian neoplasms in dogs was investigated; the differential frequency between epithelial and granulosa-theca cell tumors was about the same as seen in women. Two pure breeds were identified with significantly excessive risk; mongrel dogs were spared from malignant tumors, but had about the same risk for benign tumors as purebred dogs.

Canine testicular tumors were studied; three cell types predominated with about equal representation (seminoma, Sertoli cell and interstitial cell tumors). Several pure breeds were identified with excessive risk for one or more cell types. Male dogs with an inguinal hernia had 5 times greater risk for testis tumors than dogs without the defect. Cryptorchism increased the risk for testis tumors 14 fold over normal male dogs.

The epidemiologic features of perianal gland tumors in dogs were described. They suggest etiologic factors similar to those responsible for hormone-related neoplasms occurring in human beings.

An-androgen related skeletal herniation (perineal hernia) in dogs was reported. Therapeutic evidence indicates herniorrhaphy accompanied by orchiectomy reduces subsequent visits for re-herniation.

Arsenical poisoning in domestic animals was investigated. Acute intoxication is the most common form of arsenic poisoning documented in the dog, and generally is diagnosed in pups 2 to 6 months of age.

A study of congenital hydrocephalus in dogs indicates size of the breed is negatively correlated with risk, that is, the smaller the breed, either in weight or height, the greater the risk for the condition.

Significance to Biomedical Research and the Program of the Institute: Experiments on animals and the use of knowledge about natural occurring disease in animals are essential to progress in medical research.

The reason for the male predominance of human renal carcinoma is obscure. Environmental factors, such as tobacco and occupational exposures, have been suggested. However, these factors cannot account for the male excess of renal cancer in dogs. The risk in dogs suggests host susceptibility including the endogenous production or metabolism of sex hormones. The dog appears a suitable model for further clarification of the etiology of renal cancer in humans.

The Pointer breed was identified with a familial predisposition for ovarian epithelial neoplasms and ovarian malignancies irrespective of histologic cell type. This breed also expresses a significantly high risk for mammary cancer. Cytogenetic studies of this breed may shed light on the mechanisms responsible for aggregations of ovarian and mammary cancer commonly seen in human families.

Factor(s) responsible for a familial risk for canine testicular tumors expresses itself for only one cell type in some breeds, two cell types in other breeds, and three cell types in the Boxer breed. An opportunity now exists to investigate why more than one cell type is present in half of the familial aggregations of human testicular neoplasms.

Canine perianal gland neoplasms occur 6 times more often in male than female dogs. Yet, both sexes of the Cocker Spaniel breed exhibit risk for the tumor. Factor(s) responsible for this phenomenon could yield clues to the etiology of hormone related tumors in women and men (i.e. familial mammary cancer).

Proposed Course of Study: Data about environmentally influenced cancer will be analyzed for associations which may offer sentinels of low-dose hazards in man.

Publications

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04412-02-EEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogenic Effects of Therapeutic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
OTHER:	J.F. Fraumeni	Chief, Environmental Epidemiology Branch	EEB NCI

COOPERATING UNITS (if any)
Department of Epidemiology, Harvard School of Public Health
Department of Epidemiology Johns Hopkins University School of Public Health
Drug-Induced Cancer Unit
~~Kaiser Foundation Research Institute, Portland, Oregon~~

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1.5	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the long-term health effects of therapeutic drugs as they may relate to carcinogenicity. Cohort studies of exposed groups are conducted, as well as case-control studies of selected cancer sites which involve lifetime drug use histories. Emphasis in the past year has been on the evaluation of various types of estrogenic preparations and immunosuppressive drugs.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To study the long-term effects of therapeutic drugs in humans in order to identify drugs affecting risk of malignancy, and the characteristics of these risks. (2) To review what is known about the carcinogenic potential of drugs, in order to identify those requiring study in humans.

Studies and Methods Employed: (1) A retrospective cohort study of 1,891 women treated with conjugated equine estrogens for the menopause. This is a study of women so treated sometime between 1940 and 1969 and followed-up through the end of 1972. This is being done in collaboration with a gynecologist at the University of Louisville and investigators in the Department of Epidemiology at the Harvard School of Public Health.

(2) A prospective cohort study of oral contraceptive users, also done in collaboration with investigators at the Department of Epidemiology at the Harvard School of Public Health.

(3) A case-control study of cancer of the uterine corpus. This is a chart-review study of endometrial cancer patients and a control group of women having undergone a hysterectomy for a benign condition. Information obtained relates to known or suspect risk factors (parity, weight, hypertension, diabetes) and a particular emphasis on previous use of estrogenic hormonal preparations.

(4) Study of the long term effects of immunosuppressive drugs (described in detail in Project No. ZO1-CP-04401-03-EEB, "Immunologic Factors in Cancer Etiology").

(5) A case-control study of endometrial cancer in Baltimore. This study is done in collaboration with investigators at the Johns Hopkins University Medical School and School of Public Health (Contract No. NO1-CP-61023). In this study incident cases of endometrial cancer over a three year period are interviewed and histories obtained concerning risk factors for this cancer, and the use of estrogenic drugs. Similar information is obtained from 2 control individuals, one from the gynecology service and one from another service at the same hospital at which the case was diagnosed. The study is designed to investigate possible links between estrogenic preparations and the risk of endometrial cancer. Built into this study is an extensive review of the pathology of each case by two respected gynecologic pathologists who are ignorant of the exposure status of the patient when they review the slides.

(6) Two interrelated studies of the risk of a second malignancy among patients with ovarian cancer. In the first study, a group of 13,309 ovarian cancer patients treated prior to the era of aggressive alkylating agent therapy were studied by the retrospective cohort method. Their cancer experience was compared to that expected, based on rates in the general population. In the second study, a group of 5,455 patients with

ovarian cancer and treated by members of the Society of Gynecologic Oncology were analyzed also by the retrospective cohort method. Most of the patients in this group received aggressive alkylating agent therapy. Again, the cancer experience of this group was compared to that expected based on the rates prevailing under the general population.

Major Findings: (1) The use of conjugated estrogens as replacement therapy in menopausal women is associated with an excess risk of endometrial cancer that is related both to the strength of the medication used and its duration of use. An excess risk of ovarian cancer is also present in these patients, although restricted to that subgroup of women who also received DES. There is a deficit of other incident tumors in this group of women, attributable mainly to a deficit of colo-rectal cancers and other tumors within the gastrointestinal system.

(2) Overall, there was a 30% excess of breast cancer among women receiving menopausal estrogens compared to that expected. The risk increased with follow-up duration, progressing to 2-fold after 15 years. Higher risk accrued to women using higher-dose tablets and those taking the medication on an other-than-daily basis. In addition, after 10 years of follow-up observation, 2 factors related to low risk of breast cancer, multiparity and oophorectomy, were no longer so related. Estrogen use was related to an especially high risk of breast cancer among women in whom benign disease developed after they had started the drug.

(3) The use of oral contraceptives varies with social and reproductive characteristics that are risk indicators for many diseases. Oral contraceptive use was associated with increased risk of hospitalization for thromboembolic disease (relative risk = 1.5) and for mental illness, hyperthyroidism, hypertension and cancer of the uterine cervix. Oral contraceptive users were hospitalized for many non-life threating conditions 20 to 40% more frequently than were nonusers.

(4) In a group of 205 women with endometrial carcinoma, compared to a group of 205 women who had had hysterectomy for benign disease, the relative risk of endometrial cancer associated with use of conjugated estrogens was 3.1. Use of other forms of systemic estrogens showed similar elevations in relative risk. Relative risk was related to duration of dose progressing to an 11.5-fold greater risk for those using it for 10 years or more. Risk was also related to the strength of medication. The relative risk for users of the 1.25 mg. tablets was 12.7 as compared to a 2 to 4-fold relative risk among users of lesser strength tablets.

(5) Use of immunosuppressive drugs by kidney transplant recipients is associated with a 25-fold excess risk of lymphoma, that appears within the year of transplantation (See Project No. Z01-CP-04401-03-EEB for a more complete description).

(6) The relative risk of leukemia among patients given alkylating agent therapy for ovarian cancer was 36.1 and rose to 171.4 for those patients surviving the first 2 years of follow-up. No excess risk of leukemia was

noted among ovarian cancer patients treated prior to the use of alkylating agent therapy. Among survivors of ovarian cancer, excesses were also noted for cancers of the breast, uterine corpus, and colon. These excesses appeared to be independent of the therapy given. Excess risk of lymphomas, soft-tissue sarcomas, and cancers of the bladder were also noted and appeared to be attributable to radiation therapy given in the treatment of the ovarian malignancy.

Significance to Biomedical Research and the Program of the Institute: Drug exposure has been one of the most fruitful areas for identification of carcinogens in man and subsequent opportunities for preventive programs and insights into the biologic mechanisms in cancer etiology. In addition, the studies of the long-term carcinogenic effects of anti-tumor drugs is an important part of the evaluation of the efficacy of treatment of various malignancies with these agents.

Proposed Course of Project: (1) The retrospective cohort study of menopausal estrogen users will be continued by doing an overall mortality analysis and analyses of D and C's,acute coronary events, and gallbladder disease. In addition, efforts will be made to bring the follow-up information up-to-date by obtaining information on incident cancer cases and on mortality from all causes for the cohort through the end of 1976. (2) The relationship between a number of different drugs, including menopausal estrogens, oral contraceptives, anti-hypertensives, and thyroid medication and the risk of breast cancer will be analyzed from case-control information abstracted from the records of the Portland/Kaiser Health Plan. Several additional studies of the relationship between drugs and specific malignancies will be attempted within this same pre-paid system over the course of the next year. (3) Another evaluation of the relationship between a number of drugs and the risk of breast cancer and benign breast disease will be attempted by conducting a case-control interview study done in conjunction with the cases identified from the breast cancer detection demonstration projects. (4) A major attempt will be made to establish a systematic system for monitoring the long-term toxic effects (including carcinogenicity) of a number of the chemotherapeutic agents used in the treatment of specific cancers. This will be done in collaboration with the Division of Cancer Treatment and the various collaborative clinical trails which this Division funds. In the initial period, attempts will be made to evaluate the risk of second primaries among several of the clinical trials which have been in existence for some time, and which have involved exposure to single or multi-modality treatment programs for which there is substantial suspicion on biologic grounds of possible long-term carcinogenic toxicity. (5) The collaboration with Johns Hopkins University will enter the analysis phase in attempting to assess the carcinogenic potential of estrogens to the endometrium. Particular emphasis is being placed on "blind" staging and grading of the tumors involved.

Publications

Hoover, R., Gray, L.A., Cole, P., and MacMahon, B.: Menopausal estrogens and breast cancer. N. Engl. J. Med. 295: 401-405, 1976.

Hoover, R., Bain, C., Cole, P., and MacMahon, B.: Oral contraceptive use: Associations with frequency of hospitalization and chronic disease risk indicators. Am. J. Public Health. In press.

Gray, L.A. , Christopherson, W.M. and Hoover, R.: Estrogens and endometrial carcinoma. Am. J. Obstet. Gynecol. 49: 385-389, 1977.

Reimer, R.R., Hoover, R., Fraumeni, Jr., J.F., and Young, R.C.: Acute leukemia after alkylating agent therapy of ovarian cancer. N. Engl. J. Med. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04479-01-EEB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Analytic Studies in High Risk Areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Blot	Health Statistician	EEB NCI
	J. F. Fraumeni, Jr.	Chief	EEB NCI
	R. Hoover	Head, Environmental Studies Section	EEB NCI
	T. J. Mason	Health Statistician	EEB NCI
	B. J. Stone	Cancer Expert	EEB NCI
	L. Pickle	Staff Fellow	EEB NCI
	L. Brinton	Research Assistant	EEB NCI

COOPERATING UNITS (if any)

Center for Disease Control
Environmental Protection Agency
National Center for Health Statistics

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.25	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to identify and describe the environmental determinants of cancer, particularly within areas of high cancer risk in the U.S., through the use of analytic epidemiologic techniques. Toward this end, the Branch has initiated a series of case-control studies designed to test a number of hypotheses regarding the origins of cancer. Begun this year and presently ongoing are studies of lung cancer in coastal Georgia, colon cancer in rural Nebraska, and liver cancer in Southeast Texas, areas of exceptionally high risk for these tumors. In addition surveys of death certificates for statements on occupation from selected counties revealed increased relative risks of lung cancer associated with the wood/paper industry in rural, but not urban, Georgia coastal counties and of nasal cancer among furniture workers in North Carolina.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To identify and describe the environmental determinants of cancer in the United States, particularly through epidemiologic studies in areas at high cancer risk.

Methods employed: After U.S. counties with high cancer rate have been identified through descriptive (e.g., the cancer maps) or correlational studies, local studies are undertaken to investigate possible explanations. As a first step, death certificates are obtained and examined for information on occupation, residential address, duration of stay within the county, and contributing causes of death. Differences in these factors between cancer and other deaths are sought. These investigations are followed by case-control interview studies, where incident cancer cases and controls (or their next-of-kin) are interviewed concerning lifetime histories with respect to a number of variables including occupation, smoking, diet, medical conditions, and environmental exposures. If indicated, suggested associations from the case-control studies are followed up by cohort (prospective) studies where groups of individuals exposed to potential carcinogens are identified and their cancer experience evaluated. Laboratory and environmental measurements would then follow to attempt to pinpoint carcinogenic agents.

Major Findings: Copies of death certificates for cancer and other causes were obtained from several areas of the U.S. in which mortality rates were unusually high. Statements on occupation, industry, place of birth and duration of residence were then compared. In this way a 4-fold excess risk of cancer of the nasal cavity and sinuses was observed among furniture workers in North Carolina counties where that industry is heavily concentrated. A link between woodworking and nasal adenocarcinoma had been demonstrated in several countries, but this was the first indication that a similar hazard may exist within the United States.

A death certificate comparison for lung cancer in coastal Georgia showed higher frequencies of construction, wood/paper, and chemical (broadly defined) workers among cases than controls. The higher risk among wood/paper employees was limited to rural counties where it exceeded 3-fold. Preliminary tabulations of lung cancer death certificates from Florida pulp and paper mill counties, however, show only a small increased relative risk among mill workers.

A case-control interview study to determine factors responsible for the exceptionally high lung cancer occurrence among residents along the southeast Atlantic coast of the U.S. was begun in the fall. Incident lung cancer cases, 1970-76, and controls were identified from hospital records in the coastal Georgia city of Brunswick. Interviews of approximately 150 cases and 300 controls (or their next-of-kin) for lifetime smoking, occupation, and residence histories were completed by spring. Local cooperation was excellent, and the field team achieved a remarkable participation rate of 98%, with only 10 interview refusals. The data are now undergoing computer edit. Preliminary indications are that their completeness is quite good; e.g., the information from the interviews on cigarette smoking allows estimates of pack-years of use for

over 90% of the study group. Initial tabulations show the expected dramatic increase in risk with cigarette use.

Significance to Biomedical Research and the Program of the Institute: These studies allow us to test hypotheses regarding the etiology of cancer in the United States. Answers obtained may lead to the recognition of cancer hazards, and may directly suggest actions that need be taken to reduce or prevent the exceptional rates of cancer occurrence in certain areas of the country.

Proposed Course of Project: Other field projects in high-risk areas of the U.S. are planned. These include case-control studies to evaluate the possible relationship between textile occupations and oral cancer in North Carolina; between automotive work and bladder cancer in and around Detroit; and between shipbuilding and lung cancer in Bath, Maine. An investigation to determine reasons for the high risk of bladder cancer among both sexes in rural Vermont, and the increased risks of esophageal cancer among blacks in Washington, D.C. and Charleston, S.C. are also being designed. Analytic surveys of breast cancer among pre- and post-menopausal women to assess the relative roles of fertility patterns, drug use, body size, and diet in these two age groups are being planned.

Further death certificate surveys are also beginning. Certificates have been requested for liver and gallbladder cancer deaths from West Virginia, Minnesota, Ohio, Michigan, Wisconsin and Texas; for bladder cancer deaths from Wisconsin, Illinois and Iowa; and for oral cancer from Missouri.

Collaborative studies with other governmental agencies (CDC, NIOSH, EPA, NCHS) will be continued and expanded, so that a coordinated approach to field studies of cancer in the U.S. will be established with each agency contributing according to its strengths.

Publications

Brinton, L.A., Blot, W.J., Stone, B.J., and Fraumeni, J.F., Jr.: Nasal cancer among woodworkers in North Carolina: A death certificate analysis. Cancer Res. In press.

Harrington, M., et al: Lung cancer in coastal Georgia: Death certificate analysis of occupation. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CP-04480-01-EEB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of Occupational Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Decoufle	Health Statistician	EEB NCI
	A. E. Blair	Epidemiologist	EEB NCI
	T. L. Thomas	Sociologist	EEB NCI
	D. J. Grauman	Systems Analyst	EEB NCI
	T. J. Mason	Health Statistician	EEB NCI

COOPERATING UNITS (if any)
Social Security Administration

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Field Studies and Statistics

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.25	3.75	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns identification of unusual patterns of cancer within specific occupational groups that may be indicative of exposure to hazards in the work environment. Study groups are selected for a variety of reasons including 1) known or potential exposure to established or suspect carcinogenic substances, 2) prior case reports and case-control studies suggesting an unusual cancer experience for the group, 3) availability of existing sources of data for defining a population-at-risk. Data sources include employment and death records maintained by companies, membership and death listings maintained by labor unions and professional organizations, and state licensing records. Studies underway include cohort mortality studies of persons employed in dry cleaning establishments, men employed in different work areas of large manufacturing plant (machinists, foundry workers, welders, platers), and persons exposed to benzene and other chemicals in a single chemical production plant. Proportionate mortality studies are being conducted for workers in the petrochemical industry and pharmaceutical and biological manufacturing, chromate painters and leather workers.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: Identification of occupational groups at high risk of cancer at specific sites and carcinogenic materials to which they may be exposed.

Methods Employed: Cancer mortality patterns are determined through long-term follow-up of persons previously employed in specific plants, industries, occupations, and companies. Proportionate mortality is analyzed by cause in a set of deaths all of whom shared a common employment experience. Follow-up methods for cohort studies include the Social Security Administration, state bureaus of motor vehicle registration and drivers' licensing, state vital statistics records, and post office change of address notices. Comparisons are usually made between the cancer mortality experience of a study group and the general population (geographically specific, if possible). In some instances, internal comparisons are made with a non-exposed subgroup.

Major Findings: Most of the effort for this project has gone into initiation of specific studies which involves: determination of occupational groups deserving study, identification and evaluation of existing data sources, and securing cooperation of the person having control over the data. Contacts have been made with industrial firms, labor unions, professional organizations, state agencies, and the Social Security Administration. As a result of these efforts the following studies have been initiated: (1) cohort study of persons employed in dry cleaning shops (1940's to present); (2) proportionate mortality study of petrochemical workers (1940's to present); (3) proportionate mortality study of persons employed in pharmaceutical and biological manufacturing; (4) proportionate mortality study of chromate painters; (5) proportionate mortality study of leather workers; and (6) cohort study of workers exposed to benzene and other chemicals. Continuing analysis of cause-specific mortality rates in a cohort of men employed in a large manufacturing plant is focusing on detailed cancer sites for persons exposed to cutting oil mist, foundry workers, welders, and platers. These analyses extend an initial report on cancer mortality among workers exposed to cutting oil mist that showed moderate elevations in respiratory and digestive cancer mortality after a latency period of 30 years. Recent analysis suggests that cancers in one or two specific digestive organs may account for the previous observations.

No major findings are available on the other studies since they were only recently initiated.

Significance to Biomedical Research and the Program of the Institute: Studies of the cancer experience of working populations have provided much of the information known about chemical carcinogenesis in man. Occupational groups may be regarded as indicators in evaluating possible hazards to the general population, since exposures are generally heavier and well defined, and carcinogenic response is usually associated with a shorter latency period.

Proposed Course of Project: An increasing number of industrial populations will require epidemiologic study as leads are developed from clinical and laboratory observations. It is intended to become involved in additional studies of occupational groups to assess their cancer risks. In addition to evaluating cancer

risks among exposed workers, studies of possible mutagenic and teratogenic effects may be planned. These would elicit information on pregnancy outcomes (miscarriages, fetal deaths, congenital malformations) among women occupationally exposed to potentially carcinogenic substances, and wives of male workers in similar work environments.

Publications

Decoufle, P.: Cancer Risks Associated with Employment in the Leather and Leather Products Industry. Arch. Environ. Health. (In Press).

Decoufle, P., Lloyd, J.W., and Salvin, L.G.: Causes of Deaths Among Construction Machinery Operators. J. Occup. Med. 19: 123-128, 1977.

Lloyd, J.W., Decoufle, P., and Salvin, L.G.: Unusual Mortality Experience of Printing Pressmen. J. Occup. Med. (In Press).

Decoufle, P., Lloyd, J.W., and Salvin, L.G.: Mortality By Cause Among Stationary Engineers and Firemen. J. Occup. Med. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04481-01-EEB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Radiation-Induced Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles E. Land	Mathematical Statistician	BB	NCI
OTHER:	John D. Boice	Epidemiologist	EEB	NCI
	William J. Blot	Health Statistician	EEB	NCI
	Joseph Scotto	Demographer	BB	NCI
	Betty J. Stone	Mathematician	EEB	NCI

COOPERATING UNITS (if any)

Radiation Effects Research Foundation
Bureau of Radiological Health

LAB/BRANCH
Environmental Epidemiology Branch, FSS Program, DCCP

SECTION

INSTITUTE AND LOCATION
NCI,NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to examine cancer incidence and mortality among populations exposed to varying amounts of ionizing and non-ionizing radiation. Groups from which useful data can be obtained include the Japanese A-bomb survivors, patients exposed to high-dose medical diagnostic radiation and to therapeutic radiation, workers with histories of occupational exposures to radiation, and the populations of SEER reporting areas with different levels of solar UV radiation. The listed project members all have individual research interests in radiation-induced cancer. One of the aims of establishing this new project is to provide a framework for increased interaction and cross-fertilization of ideas.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To examine cancer incidence and mortality in populations exposed to ionizing and non-ionizing radiation, to identify specific radiation-caused cancers, and to characterize the nature of their dependence on radiation dose and other factors.

Studies and Methods Employed:

1) Studies of Japanese A-bomb survivors. These studies utilize the resources of the Atomic Bomb Casualty Commission (ABCC) and its successor organization, the Radiation Effects Research Foundation (RERF), and are based on the Life-Span Study (LSS) sample of 82,332 A-bomb survivors and 26,542 non-exposed controls resident in Hiroshima and Nagasaki in 1950. Several of these studies were begun while the participating project members were employees of RERF, and others utilize intermediate results of these studies or published ABCC reports.

a) An evaluation of the accuracy of death certificate diagnoses, which form the basis for ABCC-RERF mortality studies, was completed and published.

b) The analysis of mortality by cause of death during 1950-1974 was carried through its final stages and a report was prepared for publication. Autopsy and tumor registry incidence data were also analyzed.

c) Analyses of female breast cancer incidence data for 1950-1969 resulted in the preparation of two manuscripts for publication.

d) The temporal distributions of leukemia, lung cancer, and breast cancer diagnoses occurring among LSS sample members and among the population of A-bomb survivors generally were analyzed for differences between high-dose and low-dose or non-exposed persons of similar ages in 1945.

e) Negotiations were pursued to initiate a collaborative study with RERF of the interactions that may exist between radiation and other host factors related to breast cancer in the LSS sample. Of particular interest is the degree to which women at high risk of breast cancer because of family or reproductive history may be particularly sensitive to radiation-induced breast cancer.

2) Studies of patients with histories of medical exposure to radiation.

a) A cooperative study has been initiated with investigators of breast cancer incidence in women exposed to multiple chest fluoroscopies and to x-ray therapy for acute postpartum mastitis, to see if these data can be combined with breast cancer incidence data on Japanese A-bomb survivors to provide improved estimates of risk of radiation-induced breast cancer for U.S. women.

b) Project members have been active in a group of FS&S staff organized for the purpose of evaluating data on risks and benefits associated with mass screening for breast cancer of asymptomatic women by mammography.

c) A follow-up study of thyroid tumor incidence among patients exposed during childhood to 131-iodine thyroid uptake tests, and of matched hospital and sibling controls, has been initiated by interagency agreement with the

Bureau of Radiological Health (BRH). The study is to be carried out by the BRH with participation of members of this group, and will require 4 - 5 years.

3) Studies of patients with histories of occupational exposure to ionizing radiation.

Project members were requested by ERDA to review critically a proportional mortality study of cancer and radiation dose among workers at the Hanford Plutonium Works. This review included a reanalysis of the data by adjusting for possible confounding of cumulative dose with age at death and year of death.

4) Studies of populations exposed to non-ionizing radiation.

An ongoing study of metered incident solar UV-B radiation and non-melanoma skin cancer incidence in the SEER reporting areas was expanded to include more reporting areas and to include information on skin complexion and other host factors that may be related to susceptibility.

5) Consultant activities and service on expert committees.

Project members have served as consultants or committee members for the National Council on Radiation Protection, the Energy Research and Development Agency, the Environmental Protection Agency, the National Academy of Sciences-National Research Council's Advisory Committee on the Biological Effects of Ionizing Radiation, and the interfederal Biological and Climatic Effects Research Program task force.

Major Findings:

1) Studies based on death certificate diagnoses for members of the LSS sample suffer from the underdiagnosis of certain cancers in the elderly, particularly those who do not die in hospitals. Lung cancer and cancer of the uterine cervix are primary examples.

2) New cancers have been added to the list of those caused by the ionizing radiation from the 1945 A-bombs. These are the lymphomas and cancers of the esophagus, stomach, and urinary organs. In addition, suspicion is cast on other cancers, especially cancers of other digestive organs. The effect of age at exposure is strong, but varies according to site. Solid tumors, as a group, now outweigh leukemia as a radiation effect in terms of absolute risk. In general, the radiation effect appears stronger in Hiroshima, suggesting that neutrons are more effective as carcinogens than gamma rays. Cancer is clearly the major late effect of the A-bomb radiation on mortality.

3) The dose response for breast cancer appears to be approximately linear at low doses, and is established at doses as low at 17 rads. Neutron and gamma ray radiation appear equally effective, in contrast to many other cancers. Latency seems unrelated to dose, those irradiated having more breast cancer

but with the same age pattern at diagnosis as unirradiated women. There is evidence of breast cancer resulting from irradiation after menopause.

4) While leukemia has exhibited a dose-dependent "wave" of increased incidence, depending upon age at exposure as well as dose, breast and lung cancer show no such pattern. It is suggested that the mechanisms of carcinogenesis are fundamentally different, that the two solid tumors require age-related events, in addition to radiation, before they can occur clinically.

Significance to Bio-medical Research and the Program of the Institute:

Information about the carcinogenic effects of radiation is required in order to minimize risk while taking advantage of its benefits in medical, economic, and recreational activities. Epidemiological data relating cancer incidence and mortality to radiation exposure, especially with good information on dose and timing of exposure, can influence theories of carcinogenesis and motivate useful experimental research.

Proposed Course of Project:

The activities described here were the results of the individual activities of project members reflecting their individual interests and professional connections. Since their interests are similar, the project members may benefit from each other's knowledge, enthusiasm, and access to different sources of information. The project will continue as described above.

Publications

Boice, J.D. and Monson, R.R.: Breast Cancer following repeated examinations of the chest. J. Natl. Cancer Inst. Publication scheduled for September 1977.

Steer, A., Land, C.E., Moriyama, I., Tamamoto, T., Asano, M. and Sanefuji, H.: Accuracy of diagnosis of cancer among autopsy cases: JNIH-ABCC population for Hiroshima and Nagasaki. Gann 67: 625-632, 1976.

Land, C.E., McGregor, D.H.: Temporal distribution of risk after exposure. Proceedings of the Third International Symposium on Detection and Prevention of Cancer. Vol. I, Chapter 8: Radiation-Induced Cancer. Marcel Dekker, NY, 1977. In press.

McGregor, D.H., Land, C.E., Choi, K., Tokuoka, S., Liu, R.I., Wakabayashi, T. and Beebe, G.W.: Breast cancer incidence among atomic bomb survivors, Hiroshima and Nagasaki, 1950-69. J. Natl. Cancer Inst. Publication scheduled for September 1977.

BOSTON UNIVERSITY MEDICAL CENTER (NO1-CP-71029)

Title: A Case-control Study of Carcinoma of the Endometrium in Relation to Lifetime Exposure to Exogenous Female Hormones.

Contractor's Project Director: Dennis Slone, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To assess the risk of endometrial cancer associated with the use of drugs containing female hormonal preparations, primarily estrogens.

Methods Employed: This is a multi-institutional case-control study. The cases will be endometrial cancer cases diagnosed in hospitals in Boston, Kansas City, and Tucson which are also participating in the large FDA-sponsored surveillance of adverse drug reactions. Four controls will be selected for each case, matched to the case for age and race. Two of these controls will be women hospitalized for acute benign conditions and will have been obtained in the routine collection of information for the FDA study. The two other controls will be specifically selected as women with other cancers, excluding those of the breast and female genital tract. Lifetime history of female hormone use, for a variety of indications, will be obtained for both cases and controls, as will information relating to a number of potential confounding factors. The pathology specimens from the cases will be submitted to a pathologist for his review without knowledge of exposure status. In situ cancers will be excluded from this study.

Major Findings: This contract has recently been initiated. Data are being collected and analyzed.

Significance to Biomedical Research and the Program of the Institute: Drug exposure has been one of the most fruitful areas for identification of carcinogens in man and subsequent opportunities for preventive programs and insights into biologic mechanisms in cancer etiology. The current massive exposure of the population to steroid hormones also mandates the appropriate evaluation of the long-term health effects of this practice, particularly in view of recent findings associating these agents with excess risk of endometrial cancer. Two points need particular emphasis: (1) Information on a large enough number of cases so that risk assessments could be made specific for some of the more advanced stages of this cancer; and (2) Large enough numbers need to be assessed in order to evaluate the influence of other agents (oral contraceptives) and other therapeutic regimens.

Proposed Course of the Project: The methods employed during this year will be continued.

Date Contract Initiated: May 1, 1977

Current Annual Level: $35,478

BUREAU OF THE CENSUS (YO1-CP-70501)

Title: County Population Estimates by Age, Race, and Sex for the 1970's

Principal Investigator: Richard Engels, Assistant Chief, Populations Division

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This agreement covers the experimental development of estimating methodology and the preparation of estimates of the resident population for all United States counties by age, race, and sex for each year, 1970 through 1975 (July 1).

Methods: The county units provided will either be identical with the units used by the National Cancer Institute in its publication of 1950-70 mortality data, or subsets thereof. The estimates will be by age (5-year groups to 85 years and over), cross-classified by sex and race (two groups; White, and Black and Other Races). The initial model design will produce estimates for the age groups 65-69, 70-74, and 75 and over, and if the data prove to be of acceptable quality, the age range will be extended accordingly.

Major Findings: The contractor is making excellent progress on this project. It is estimated that the work will be completed by mid-summer 1977.

Significance to Biomedical Research and the Program of the Institute: There is no readily available source of population estimates at the county level for the time period in question, 1971 through 1975. These populations are vital to the continuance of investigations concerning the geographic distribution of malignancy in the United States which the Environmental Epidemiology Branch has developed over the past several years.

Proposed Course of Project: This project represents the first step in a program designed to produce annual population estimates on a regular basis in the detail specified, so as to meet the needs of the National Cancer Institute. A continuing relationship with the National Cancer Institute in reviewing and improving the estimating methodology is foreseen, and the Bureau of the Census is engaged in a cooperative effort with State agencies to improve the methodology and strengthen the estimates.

Date Contract Initiated: January 10, 1977

Current Annual Level: $80,000

BUREAU OF RADIOLOGICAL HEALTH (YO1-CP-70506)

Title: Follow-up Studies in Cancer: Childhood Exposure to ^{131} Iodine Thyroid Uptake Tests.

Contractor's Project Director: Frank E. Lundin, M.D.

Project Officer (NCI): Charles E. Land, Ph.D.

Objectives: This is a follow-up study of 6500 patients given ^{131}I thyroid uptake tests at age 15 or younger during the period 1946-1967. The primary study objective is to determine whether any long-term radiation effects, thyroid cancer in particular, are associated with these radiation exposures.

Methods Employed: In addition to the 6500 exposed patients, sibling and hospital controls in equal numbers are also to be selected and followed. Follow-up is to be by mail questionnaire, except that in the case of patients no longer living, death certificates will be obtained and causes of death validated through hospital records.

Major Findings: Research results are not yet available, as this contract was initiated on May 1, 1977.

Significance to Biomedical Research and the Program of the Institute: Follow-up studies of persons with histories of thyroid exposure to ionizing radiation have established that increased incidence of benign and malignant tumors is a long-term radiation effect. Exposure during childhood appears to have more effect than comparable exposures during adulthood. While most of the data pertain to external sources of radiation, a greatly increased incidence of thyroid nodules was observed in Marshallese children who ingested radioactive iodine from H-bomb fallout, with consequent very high doses to the thyroid from internal beta-particle emitters. There is some evidence that the tumorigenic effect per rad from internal beta-particle emitters is less than that from external sources. The use of the isotope ^{131}I as a diagnostic tool for medical examination of the thyroid represents a current possible hazard to tested patients, and has created a population of exposed individuals for which this hazard can be evaluated.

Proposed Course: During this year subjects will be located so that follow-up by mail questionnaire can proceed at a later date. In the case of subjects no longer living, death certificates will be obtained and causes of death validated through hospital records.

Date Contract Initiated: May 1, 1977

Current Annual Level: $100,000

UNIVERSITY OF HAWAII (NO1-CP-71006)

Title: Occupational Cancer Risk in Hawaii

Principal Investigator: Ming Pi Mi, Ph.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This project is designed to provide data which will permit the linkage of official records: Civil Defense file, birth records and death certificates for the population of Hawaii from 1942-1975 with specific emphasis on occupation and/or place of employment.

Methods Employed: From a Civil Defense file with detailed information on the Hawaiian population of 1942-43 occupational data will be abstracted for the Oahu cohort. New data to be added to the computer file would include all information on individuals residing in the remaining counties (islands of Kauai, Maui, Molokai, Lanai and Hawaii) during the war years. These data are important because of the number of individuals who resided and worked on sugar, pineapple and rice plantations. Occupational data will be abstracted from birth records which give occupation of parents. Comparable data will be abstracted from all death certificates for the period in question.

Major Findings: There are none to date since the contract was awarded April 11, 1977.

Significance to Biomedical Research and the Program of the Institute: The collection of occupational data fits in well with the specific interest in the geographic distribution of malignancy in the United States that the Environmental Epidemiology Branch has developed over the past several years. The ability to link records over this time span will greatly facilitate the investigation of occupational factors related to malignancy.

Proposed Course of Project: The resultant record-linked data bases will permit the selection of a final occupational group of interest from death certificate data and the linkage of official records on these individuals through time using birth records as interim checks for changes, and Civil Defense records for an initial occupation.

Date Contract Initiated: April 11, 1977.

Current Annual Level: $66,849

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-43201)

Title: Follow-up of Children Who Received Very Early in Life Polio Vaccine Inadvertently Containing SV 40

Contractor's Project Director: Edward A. Mortimer, Jr., M.D.

Project Officer (NCI): George J. Burton, Ph.D.

Objectives: The purpose of this study has been to determine whether any deleterious effects, particularly oncogenesis, occur in individuals who were inoculated orally or intramuscularly very early in life with polio vaccines inadvertently containing varying amounts of viable simian virus 40 (SV 40). The study comprises an annual follow-up of 1,073 children who received such poliomyelitis vaccines in the newborn period during the years 1960 and 1962 at Cleveland Metropolitan General Hospital and University Hospital of Cleveland.

Methods Employed: Attempts are made to determine the location of each child twice annually, through telephone contact where possible, contact with parents, guardians, or relatives, review of rosters of boards of education in the Cleveland area, Cleveland area post office checks, Ohio Bureau of Vital Statistics, Bureau of Motor Vehicles checks regarding drivers' licenses, checks with the Welfare Department, telephone listings including telephone books for out-of-state communities, Social Security records, contacts with neighbors, neighborhood visits, examination of clinic and hospital records, and in some cases contacts with medical colleagues. In an effort to establish direct contact, a brief letter was sent to the parents of each study subject for whom an address was available so as to try to determine current health status and to establish contact for future study purposes. Deaths are also discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The names in the study group are compared against an alphabetic file of all childhood cancer deaths in the U.S. maintained in the National Cancer Institute. The frequency of cancer deaths in the study group will be compared with the expected cancer mortality for children in the State of Ohio. All the data have been computerized.

Major Findings: After 15 to 17 years there have been no cancers or other disorders in the study group attributed to SV 40. There have been 15 deaths among the cohort; 3 were due to accident, and no cancers were among the other 12. The twelve non-accidental deaths were associated with congenital biliary atresia, bronchopneumonia, convulsions with complications, sepsis, erythroblastosis fetalis, acute necrotizing laryngitis, or congestive heart failure. Most of these children died before this contract was initiated, i.e., before March 1964. Fifty-nine out of 950 children (6.2%) are enrolled in special classes because of mental retardation; this, however, is not significant because the overall rate of students in special classes on the junior high school level in the city of Cleveland is 4.96%.

Of the original cohort of 1,073 study subjects, 973 (90.7%) were known to be alive during 1976 or the first four months of 1977. Of the original 1,073, 85 (7.9%) have not been accounted for or traced. A major effort has been

made to try to establish communication with the 973 study subjects or their families for whom current addresses are available. Responses have been received from only 416 (42.8%) of these during the past 16 months.

Significance to Biomedical Research and the Program of the Institute: It is known that neonatal exposure to animal oncogenic viruses is important in the induction of virally-induced neoplasms. This suggests that the cancer mortality of children inoculated with SV 40 at birth might be different from that of children exposed at school age. Non-defective Adeno-2-SV 40 hybrids are considered by the National Cancer Institute to be viruses of moderate risk for man. The oncogenic potential of SV 40 in man is based on results of laboratory and clinical experiments: (a) Undifferentiated sarcomas develop in newborn hamsters 2½ - 5½ months after SV 40 is inoculated in high doses SC, IM, IP or intrathoracically. The SC route may also yield fibrosarcomas. Intracerebral inoculation often results in brain tumors. (b) SV 40 transforms human kidney, skin, and thyroid cells. (c) Nodules, which were undifferentiated sarcomas histologically, developed and then regressed in volunteer terminal cancer patients who had received transplants of W-18VA2 (human buccal mucosa) cells transformed by SV 40 but not releasing virus. SV 40 must be differentiated from two other closely-related papova-type viruses-JC and BK viruses, which have been also considered by some virologists as potential oncogenic agents in man.

Proposed Course of Project: Although there have been no cancers among 973 known children who were neonatally inoculated with SV 40 15 to 17 years ago, this contract is being continued until a decision is reached as to how many years represent a sufficient period of follow-up. The chances of cancers appearing among this cohort is diminished by the fact that 921 (85.8%) of the 1,073 children ingested the SV 40 virus in Sabin oral polio vaccine; experimentally positive results occur in neonatal hamsters only when SV 40 is introduced in very large doses via needle-inoculation. Some latent periods for oncogenesis following exposure to carcinogenic chemicals or substances may be as long as 40 years, but it is not known whether this applies also to a virus such as SV 40.

Date Contract Initiated: March 9, 1964

Current Annual Level: $41,079

CENTER FOR DISEASE CONTROL (YO1-CP-70500)

Title: Epidemiologic Studies in Cancer

Contractor's Project Director: Clark Heath, M.D.

Project Officer (NCI): William J. Blot, Ph.D.

Objectives: To provide partial support for two field studies in cancer epidemiology: (a) a case-control study of lung cancer in coastal Georgia; (b) a case-control study in Houston, Texas designed to test whether parents of cancer cases are more heavily exposed to hydrocarbons or other industrial exposures.

Methods Employed: In the first study, 150 incident lung cancer cases and 300 controls identified from 1970-76 hospital records in Brunswick, Georgia have been interviewed regarding lifetime smoking, occupation, and residence histories. Interviews are now ongoing for lung cancer cases and controls diagnosed in the 3 hospitals in Savannah, Georgia. Coastal Georgia was selected for study since the area is at the highest risk for lung cancer of any area of the U.S. The second study involves interviews of parents of children with cancer currently being followed at the Texas Children's Hospital Research Haemotology Clinic in Houston, and 3 sets of controls.

Major Findings: The field effort in Brunswick was completed, without any major drawbacks, in a total period of less than 6 months. Local cooperation was excellent, and the field team achieved a remarkable participation rate of 98% (there were only 10 interview refusals) of the target population. The data are now undergoing computer edit. Preliminary tabulations indicate that the data are likely to be quite complete, e.g., information was available to estimate pack-years of cigarettes smoked for over 90% of the study group. Data from Savannah and Houston are not yet available.

Significance to Biomedical Research and the Program of the Institute: Field studies in areas of the U.S. at high cancer risk are likely to produce good leads to the environmental causes of cancer. From the local point of view, such studies are urgently needed to help suggest means to reduce the excessive toll in morbidity and mortality to residents.

Proposed Course of Project: Continuation of same methods.

Date Contract Initiated: October 1, 1976

Current Annual Level: $51,720

COUNCIL ON ENVIRONMENTAL QUALITY (YO1-CP-70507)

Title: Water Quality Databases and Expansion of Computer Systems

Principal Investigator: Warren R. Muir, Ph.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This agreement covers the development of two databases representing water quality data from extensive monitoring stations on drinking water sources.

Methods Employed: One database will consist of surface water stations from the STORET databank, and the other will be composed of ground water stations chosen from WATSTORE. The Council on Environmental Quality will perform detailed edit checks of the respective databases, and provide NCI with the most comprehensive database on drinking water quality available in our country.

Major Findings: The contractor is making excellent progress on the databases in question. The work to date has shown there will be no major difficulty in expanding the NCI's environmental exposure databases to include this information on water.

Significance to Biomedical Research and the Program of the Institute: The specified databases and computer system are unique, and will enable the Environmental Epidemiology Branch to pursue hypotheses concerning water-borne chemicals (some of which are carcinogenic) and malignancy.

Proposed Course of Project: These data will be used by the staff of the Environmental Epidemiology Branch in analytical studies of the associations between components of drinking water distribution systems and malignancy. The agreement also provides NCI with access to these databases on the CEQ UPGRADE system. This computerized system, which is resident at the NIH computing facility, will provide the Environmental Epidemiology Branch with cost effective access to the two databases mentioned above. In addition CEQ will supply the NCI project officer with a paper copy of the current program for UPGRADE so that NCI can evaluate its utility for other analytical purposes or for accessing other databases maintained by the NCI Environmental Epidemiology Branch.

Date Contract Initiated: May 1, 1977

Current Annual Level: $50,000

JOHNS HOPKINS UNIVERSITY (NO1-CP-61023)

Title: Epidemiologic Studies of Drug-Induced Cancer

Contractor's Project Director: Neil B. Rosenshein, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To investigate the risk of endometrial cancer associated with the use of exogenous female hormones.

Methods Employed: A case-control study is currently underway in which the patients having developed endometrial cancer within the past three years in Baltimore are being interviewed for their use of exogenous female hormones, as well as a range of other risk factors for endometrial cancer. Two control groups are also being interviewed. One is chosen from the same hospital as the case, hospitalized for any non-gynecologic condition, and the other is similarly chosen only from a group of women hospitalized for gynecologic problems. In addition, the pathological material from the cases is being reviewed by two prominent gynecologic pathologists who are unaware of the exposure status of the patient when they are reviewing the slides.

Major Findings: The interviewing is just being completed, and the questionnaires are being analyzed.

Significance to Biomedical Research and the Program of the Institute: Drug exposure has been one of the most fruitful areas for identification of carcinogens in man and subsequent opportunities for preventive programs and insights into biologic mechanisms in cancer etiology. The current massive exposure of the population to steroid hormones also mandates the appropriate evaluation of the long-term health effects of this practice.

Proposed Course of Project: Over the course of the next 6 months the information from the interview and hospital segments of this study will be computerized and analyzed.

Date Contract Initiated: February 1, 1976

Current Annual Level: $144,000

KAISER FOUNDATION RESEARCH INSTITUTE (NO1-CP-61057)

Title: Change in Breast Cancer Risk Among Estrogen Users

Contractor's Project Director: Andrew Glass, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To study the risk of breast cancer associated with the use of exogenous female hormones and other drugs.

Methods Employed: 437 cases of breast cancer diagnosed in the Portland (Oregon) Region Kaiser Permanente Medical Group between January 1969 and December 1975 comprised the study group. Two controls for each case have been chosen from a 5% sample of all members of the plan. These controls have been matched to the corresponding case by age, year of diagnosis, sex (all female), postal zip code area of residence, and duration of Health Plan membership. The outpatient medical records for each case, and for each control, are obtained and all relevant information in these records abstracted. This relevant information includes year of birth, race, height, weight, age at and type of menopause, parity, age-at-first birth, history of hypertension, history of benign breast disease, and all drugs taken while in the Kaiser Plan. Specific attention is given to estrogens, oral contraceptives, reserpine, spironolactone, thyroid extract, and the major and minor tranquilizers. Strength and duration of use of each medication are abstracted along with brand name and year first used.

Major Findings: The data abstracting and computerization process is just nearing completion. Major substantive findings await the analyses which will be performed over the course of the next several months.

Significance to Biomedical Research and the Program of the Institute: Drug exposure has been one of the most fruitful areas for identification of carcinogens in man and subsequent opportunities for preventive programs and insights into biologic mechanisms in cancer etiology. The current mass exposure of the population to steroid hormones mandates the appropriate evaluation of the long-term health effects of this practice. In addition, a study from the Environmental Epidemiology Branch which was published in the past year indicates an excess risk of breast cancer among long-term users of conjugated estrogens for menopausal symptoms. Because of the magnitude and gravity of the breast cancer problem in this country and the magnitude of exposure to these agents, a prompt testing of this hypothesis is necessary.

Proposed Course of Project: Following the analyses of the data outlined above, additional sites of malignancy for which there are therapeutic drug hypotheses or occupational hypotheses that can be tested in this system will be pursued via the case-control approach.

Date Contract Initiated: July 1, 1976

Current Annual Level: $56,731

LITTON BIONETICS, INC. (NO1-CP-61016)

Title: Immunologic Assessment of High Risk Cancer Families

Contractor's Project Director: Jack Dean, Ph.D.

Project Officer (NCI): William A. Blattner, M.D.

Objectives: To evaluate the role of host immunologic factors in the predisposition of cancer prone families to malignancy.

Methods Employed: Utilizing methods developed under a related NCI contract aimed at assessing immunologic parameters in individuals with cancer, tests which have proven useful are applied to clinically involved and clinically normal members of families with unusual aggregations of cancer.

Major Findings: Over 600 individuals from cancer prone families have been tested, and analysis of this body of data is currently in the initial phases. To facilitate this a computerized data set is being developed, so that immunogenetic questions can be evaluated. These data will be correlated with tissue typing information and other laboratory information. Several melanoma-prone families have been tested, and tumor-specific responses appear to correlate with clinical features of cancer risk in individuals with premalignant moles. Attempts to link this information to pathologic observations of immunocyte infiltrates of tumors are currently under way. A family prone to Hodgkin's disease and nasopharyngeal carcinoma was tested. Subclinical defects in immune responsiveness were confirmed in separate experiments on several clinically normal family members. These observations correlate with previous observations in families prone to lymphoproliferative disorders.

Especially interesting has been the observation in a number of families that there appear to be defects in recognition of tissue antigens of non-HL-A identical siblings. This finding may be of significance in defining subtle defects in recognition of abnormal tissue antigens as would be predicted for immune-related susceptibility theories.

Tumor tissues from members of families under study have become available through close contact with families, and are being used in a newly developed migration inhibition assay. Preliminary results suggest tumor-specific reactivity in certain families.

A family prone to colon cancer was studied in collaboration with Dr. Ivan Law. Preliminary results suggest specific immune defects that will require further confirmation in other families.

Significance to Biomedical Research and the Program of the Institute: A role for host immunogenetic factors in the etiology of cancer has been postulated on the basis of extensive laboratory studies in animals. The application of sophisticated immunologic techniques in high risk human populations offers opportunities for defining the role of host factors in the etiology of familial malignancy.

Proposed Course of Project: The Environmental Epidemiology Branch is currently undertaking a systematic study of both high risk and low risk families to evaluate the role of host immunologic factors in the etiology of familial malignancy. As new probes become available they will be incorporated into the protocols.

Date Current Contract Initiated: December 1, 1975

Current Annual Level: $121,948

MELOY LABORATORIES, INC. (NO1-CP-43216)

Title: The Use of SV 40 T-Antigen as a Potential Epidemiological Tool

Contractor's Project Director: Anthony Lubiniecki, Ph.D.

Project Officers (NCI): Joseph F. Fraumeni, Jr., M.D. and
William A. Blattner, M.D.

Objectives: To investigate the relationship of in vitro T-antigen expression in SV 40 virus-infected skin fibroblasts to the risk for developing malignancy.

Methods Employed: Based on observations of Todaro and associates, methods developed and refined under the contract are employed to measure in vitro T-antigen expression in SV 40 virus-infected human skin fibroblasts. Standardized conditions allow for reproducible measurement of T-antigen utilizing a fluorescent antibody technique developed under the contract.

Major Findings: Evaluation of a cohort of clinically normal individuals compared to 10 patients with classic features of Fanconi anemia confirms that patients with this syndrome have universally elevated T-antigen response. Parents of Fanconi anemia patients also have significantly elevated levels of T-antigen that do not differ from their offspring values. This observation argues against the widely held belief that obligate heterozygotes of Fanconi anemia have intermediate values, and suggests that a different genetic mechanism may be operative. A study of 30 families reveals that a single gene cannot account for regulation of T-antigen expression. Evidence for 3 separate and possibly additional genes was found. A study of diverse cytogenetic disorders indicates that elevated levels are shared by various syndromes suggesting a common factor for these diseases. In a related area Down's Syndrome patients and their parents were elevated, and the level of interferon production was ruled out as a significant modulator of T-antigen expression.

Significance to Biomedical Research and the Program of the Institute: The Environmental Epidemiology Branch of the NCI is concerned with studies to identify the role of host factors in the risk of cancer. This objective will be fostered by joint epidemiologic-laboratory investigations of the type employed in this contract, in collaboration with other investigators. This Branch will continue to make this laboratory resource available to other members of the research community who have patients appropriate for study utilizing this technique. The findings should be of value in clarifying the utility of this assay to identify persons at high risk for cancer.

Proposed Course of Project: The contract will be terminated on September 30, 1977 because the Field Studies and Statistics Contract Review Committee felt that a large future effort in basic biologic research, such as would be required for this project, would not be appropriate for the Environmental Epidemiology Branch. The large bank of fibroblasts gathered over the past 4 years will be made available to the Institute for Medical Research in Camden, N.J. A small professional services contract will be sought with Meloy to continue storing fibroblasts on families being studied or to be studied by the branch.

Date Current Contract Initiated: October 1, 1976

Current Annual Level: $220,224

UNIVERSITY OF MINNESOTA (NO1-CP-33357)

Title: Immunodeficiency -- Cancer Registry

Contractor's Project Director: John H. Kersey, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To study the frequency and possible determinants of malignancy in immunodeficient patients.

Methods Employed: Through the cooperation and goodwill of immunologists in many of the specialized immunology centers throughout the world, clinical information and occasionally biologic specimens from immunodeficient patients who have developed cancer are sent to the Registry for tabulation and analysis. In the past year, a concerted effort has been made to recontact all previous contributors as well as to canvass the societies of immunologists for others who may have seen such patients. The emphasis has been on ascertaining previously unreported cases of malignancy and determining the total numbers of immune deficient patients by disease category. In addition, a group of institutions has been identified which will permit closer monitoring of both immune patients seen and malignancies that develop among these patients. It is hoped that from this group reliable estimates of incidence rates among immune deficient patients can be obtained, as well as the possibility that case-control studies could be launched comparing the characteristics of immune deficient patients who developed malignancy to those of patients who did not.

Major Findings: New findings in the past year include the identification of 99 new cases. This indicates that the malignancy rate among these patients has not really declined, as might have been thought by the diminished reporting to the registry in the recent past. The diminished reporting appears to be merely an artifact of non-aggressive case finding in previous years. This marked increase in numbers of cases available for analysis should provide much better estimates of the excess risk involved. In addition, a study was done to ascertain the creditability of the diagnosis of one of these immune deficiency diseases (Bruton's agammaglobulinemia). The criteria used in diagnosing this condition seem to be quite good in the responding institutions.

Significance to Biomedical Research and the Program of the Institute: Patients with altered immunologic state have demonstrated excess risks of malignancy several hundred times that of the general population. Study of the determinants of these excesses have a high probability of shedding light on immunologic aspects of cancer etiology.

Proposed Course of Project: Aggressive case finding will continue to be pursued in order to provide larger numbers for more complete analyses. In addition, with estimates of the patients seen for each of the immune deficiency diseases and with the opportunity to follow several large clinics closely, it is hoped that reliable estimates of true incidence rates can be generated. The feasibility of conducting case-control studies of those patients developing malignancy compared to those not developing a malignancy will also be explored.

Contract No. NO1-CP-33357

Date Contract Initiated: July 1, 1974

Current Annual Level: $44,060

NATIONAL ACADEMY OF SCIENCES (NO1-CP-61003)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans

Contractor's Project Director: Seymour Jablon, M.A.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To develop and conduct a broad program of epidemiologic studies in cancer among veterans.

Methods Employed: The Epidemiology Branch of the National Cancer Institute and the Medical Follow-up Agency of the National Academy of Sciences have developed an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations of veterans ascertained through facilities of the Veterans Administration and supplemented by mortality data. During the past year efforts have focused on follow-up studies of veterans who have certain conditions that may influence the risk of cancer, such as splenectomy, allergies, alcoholism, nephrectomy, orchidopexy, and sarcoidosis. Over 6,000 veterans have been reported in the index and comparison groups, and analyses to assess the specific causes of death are nearly complete. Under review is a proposal for a study of occupational exposure to tetrachloroethane, a chlorinated hydrocarbon used for impregnating clothing against mustard gas during World War II.

Major Findings: A follow-up study of 740 servicemen splenectomized for trauma during World War II revealed excess mortality from pneumonia, ischemic heart disease, and liver cirrhosis. The findings confirm the increased risk of fatal infections with asplenia, but there was no predisposition to cancer as in some other immunodeficiency states. Postsplenectomy thrombocytosis, and hypercoagulability may account for the increased risk of fatal myocardial ischemia in this group. In contrast to reports that the allergic state may "protect" against cancer, the follow-up of servicemen with asthma revealed excess mortality from various cancers, particularly the lung and pancreas. In addition, a report is being prepared on risk factors in Hodgkin's disease among young servicemen, including an unexpected relation to small sibship size. Nearing completion is a study relating prenatal x-ray exposure to childhood cancer, with preliminary results showing a non-significant increase for leukemia and other tumors.

Significance to Biomedical Research and the Program of the Institute: The Environmental Epidemiology Branch, NCI, is concerned with studies to identify and clarify environmental and host factors in cancer. The objectives can be more readily achieved by coordinating efforts with the exceptional resources and competent staff of the Follow-up Agency.

Proposed Course of Project: The various studies will continue into the next contract year, and new projects will be identified to clarify risk factors in cancer. Emphasis will be placed on delineating occupational groups in the military that may have involved carcinogenic exposures.

Date Contract Initiated: June 28, 1971

Current Annual Level: $114,000

STATE OF NEW JERSEY DEPARTMENT OF HEALTH (NO1-CP-61031)

Title: Etiologic Studies of Cancer in New Jersey

Contractor's Project Director: Martin Goldfield, M.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: To examine the cancer mortality experience in the State of New Jersey with specific emphasis on quantifying environmental risk factors for bladder cancer.

Methods Employed: (A) Descriptive studies — A system of computer programs has been developed which with modification can efficiently calculate sex and race-specific rates for any cause of death for the period of time 1962-1975. Refinement of the data to municipalities and specific analyses to quantify biases in the approach will be pursued. (B) Case-control studies— the contractor will interview each bladder cancer case and two matched controls (or their families when appropriate) to ascertain occupational, environmental and personal characteristics of the study population. These studies will be performed in areas which have been found to have exceptionally high mortality rates, and also a state-wide study of bladder cancer cases under 50 years of age will be included.

The sources of data are death certificates, interviews of living cases and controls, occupational and industrial information from interviews of next-of-kin, and Social Security data.

Major Findings: To date the contractor's accomplishments have been considerable. Utilizing data through 1975 it has been determined that the previously detected excessive rates of bladder cancer in New Jersey for the period 1950-1969 have continued to increase in time in several counties. Utilizing moving averages, the rates for males in Cumberland and Salem counties have continued to increase. When rates were calculated for municipalities, 4 of Salem's 15 municipalities were found excessive. The municipalities cluster about one plant thought to be the employer of many cases of bladder cancer. A figure is included in the program report. These data suggest specific municipalities for future study.

Significance to Biomedical Research and the Program of the Institute: Recent reports of excessive mortality from bladder cancer in New Jersey need to be followed-up with a project such as this to assess the relative contribution to this excess from industrial (occupational), as well as common environmental exposures. This type of investigation also fits in well with the specific interest in the geographic distribution of malignancy in the United States to which the Environmental Epidemiology Branch is committed.

Proposed Course of Project: The work to date has pointed to several specific places in New Jersey which appear to warrant additional study. It is the intent of the contractor to concentrate on these areas to elicit additional

etiologic information. The need to obtain occupation and industry information from living persons is recognized and an approach has been set out. It is anticipated that the case-control investigations combined with the descriptive data compiled to date will point to specific industrial plants which should be investigated.

The objectives are threefold: (A) Descriptive studies: 1) expansion of descriptive studies to include cancers other than bladder such as colon, rectum, breast and lung; 2) continue analyses of industrial and environmental variables (water supplies, air pollution indices, etc.); 3) pursue methodologies to develop and test hypotheses concerning relationships between occupational and environmental variables and malignancy. (B) Case-control studies of bladder cancer which will develop occupational histories for the purpose of: 1) determining types of industrial exposures which are related to higher bladder cancer risk; 2) detection of specific industrial plants with excesses of bladder cancer; 3) performing appropriate analyses to measure the strength of association between specific exposure and/or personal data and bladder cancer. (C) Cohort studies within industries.

Date Contract Initiated: February 10, 1976

Current Annual Level: $83,078

THE UNIVERSITY OF TEXAS MEDICAL BRANCH (NO1-CP-71005)

Title: Epidemiology of Primary Liver Cancer in Selected Counties of Texas

Principal Investigator: Patricia A. Buffler, Ph.D.

Co-principal Investigator: Ned Snyder, III, Ph.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This project is designed to study mortality from primary liver cancer in selected counties in Texas. These counties were selected because they have the highest rates of primary liver cancer in this country.

Methods Employed: The contractor will use a case-control approach selecting two controls for each case of primary liver cancer from the next deaths which occur that match on age (within the same five-year category), sex, and race. The occupation and industry of employment will be coded from death certificates, and in addition information regarding employment and exposure in specific industries, will be ascertained.

Major Findings: There are none since this contract was first awarded on April 26, 1977.

Significance to Biomedical Research and the Program of the Institute: This project will contribute to the NCI objective of accurately assessing the incidence and mortality of cancer and identifying factors related to cancer risk. Also an investigation of this type fits in well with the specific interest in the geographic distribution of malignancy in the United States that the Epidemiology Branch has developed over the past several years. Reports of excessive mortality from primary liver cancer in Texas need to be followed up with a project such as this to assess the relative contribution to this excess from industrial (occupational), as well as, common environmental exposures.

Proposed Course of Project: It is proposed to concentrate on Brazoria County initially due to the large percent of the workforce employed in chemicals and related manufacturing (18.4%), and the fact that the Dow Chemical Co., Texas Division which is in Brazoria County has agreed to participate. Subsequent to the completion of the analysis of data for Brazoria County including a detailed review of occupational histories and available pathology, the study would be expanded to include the additional counties which have been suggested. This could take the form of occupational cohort studies if the results from Brazoria so indicate.

Date Contract Initiated: April 26, 1977

Current Annual Level: $110,261

TULANE UNIVERSITY (NO1-CP-61058)

Title: Epidemiologic Studies of Cancer in Louisiana

Contractor's Project Director: Marise S. Gottlieb, M.D.

Project Officer (NCI): William J. Blot, Ph.D.

Objectives: To identify occupational, residential, and ethnic associations with lung, bladder, pancreas, and nasopharyngeal cancer in high-risk Louisiana parishes.

Methods Employed: Death certificates for cancer cases and controls during 1962-75 in 19 parishes selected for either 1) a high cancer rate or 2) the presence of chemical, petroleum or paper manufacturing industries are being abstracted. Data are being obtained on occupation, industry, and location of residence within the parish, and also on family names and birth place in order to classify each individual according to extent of Acadian ancestry. The distribution of these variables between cases and controls will then be compared.

Major Findings: Data collection, abstracting, and computer editing for 10 parishes (the 10 smallest) are now nearing completion. Case-control comparisons are not yet available. Coding schemes for occupation and industry, adapted to the local situation, and for categorizing ancestry have been developed and can be successfully utilized for over 90% of the certificates. In addition, a characterization of source of household water supply has been developed.

Significance to Biomedical Research and the Program of the Institute: This contract provides a systematic initial investigation to discover possible reasons for the exceptionally high rates of cancers of the lung, bladder, pancreas, and nasopharynx in Louisiana parishes, providing a stepping-off point for future in-depth analytic epidemiologic studies.

Proposed Course of Project: Data collection and abstracting will be completed for the remaining parishes. Statistical analyses by Branch and Tulane staff are now beginning and will suggest avenues of needed further study.

Date Contract Initiated: July 1, 1976

Current Annual Level: $129,291

U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2, TAIWAN (Y01-CP-60502)

Title: Epidemiology of Hepatoma in Relation to Environmental Factors and to Other Liver Diseases.

Contractor's Project Director: R.P. Beasley, M.D.

Project Officer (NCI): George J. Burton, Ph.D.

Objectives: Major objectives were: (1) To determine whether persons infected with hepatitis B virus (HBV) are at increased risk of developing cirrhosis and primary hepatocellular carcinoma, and if so, to further characterize the risk factors of such carriers. (2) To determine whether hepatitis B surface antigen (HBsAg) carriers are at increased risk of developing other diseases. (3) To describe the epidemiology of hepatoma in Taiwan. (4) To determine the feasibility of studying aflatoxin in relation to hepatoma.

Methods Employed: The approach was primarily epidemiologic, with associated immunologic and serologic determinations. Three types of studies were carried out:

1. Prospective study. The first two objectives are simultaneously covered by a prospective study. Apparently healthy persons who are HBsAg-positive are identified and followed prospectively to determine their risk of developing hepatoma, cirrhosis, and chronic hepatitis. They are compared with a HBsAg-negative group drawn from the same population and matched according to sex, age, native place, and occupation. Two main study groups have been utilized: (a) 30,000 Government employees undergoing routine annual physical examinations; and (b) a cohort of 1,820 men who have been under health surveillance since 1965. These groups and their controls receive annual physical and blood examinations, including tests for HBsAg, anti-HBs, and serum transaminase. HBsAg-positive individuals will be tested for alpha fetoprotein, and resulting positives will be referred for further clinical evaluation including liver scan and perfusion to look for evidence of hepatoma. Radioimmunoassay will be used to identify HBsAg, anti-HBs, and alpha fetoprotein; and passive hemagglutination of titers.

2. Case control studies. Patients with hepatoma and/or cirrhosis, chronic hepatitis, and acute hepatitis were tested for HBsAg and anti-HBs. Cases are to be matched for age, sex, nativity, and antigen or antibody status with apparently healthy controls. Family members will be tested for antigen and antibody to determine whether there are different patterns of infection in families of persons with different kinds of liver disease, and whether they differ according to whether the index case or control is HBsAg-positive.

3. Death certificate analysis. All deaths in Taiwan require a death certificate, and certification rates approach 100%. Taiwan death certificates follow internationally established models. The data have been transcribed to Hollerith cards by the Provincial Government since 1968. Local data processing provides age-and sex-specific death rates for all counties, and cause-specific rates for Taiwan are classified according to the IDC A and B lists. Local data are not available according to the IDC three digit code for cause, nor are

there any data for cause by residence, let alone adjusted rates. The available data were borrowed, and analyzed. This year's report includes the first information derived from this evaluation.

Major Findings:

(1) Prospective study: A total of 9,904 men have been studied, 1,598 from the Cardiovascular Disease Study (CVDS) and 8,306 from the Government Employees Central Clinic (GECC). Among 1,331 CVDS men aged 50-69, 165 (12.4%) were HBsAg positive; there was a linear decline from 14.4% in the 50-53 group to 9.2% in the 65-69 group. Among the first 7,200 of the GECC group 1,098 (15.2%) were positive for HBsAg; there was a linear decline from 18.6% positive in the 40-44 age group to 11.8% in the 60-64 group.

All of China's 36 provinces were listed as places of origin among the first 7,200 GECC persons; 1,750 were from Taiwan and 5,450 from the mainland. The HBsAg+ rates were increased in a north-to-south gradient: north China origin, 9.9%; central China, 15.1%; south China, 17.6%; and Taiwan, 18.5%. Most Taiwanese are of south China origin, and hepatoma is reported to occur more frequently in south China.

HBsAg prevalence strongly correlated with a history of hepatitis (33.8% antigen+) and with a history of other liver disease (23.6% antigen+). A history of hepatitis was strongly associated with a history of other liver disease. Transaminase results correlated poorly with hepatitis and with other liver disease. HBsAg did not correlate with a history of blood transfusion or having received acupuncture.

(2) Case control study: Four hundred eight hospitalized patients with liver disease were studied - 356 males and 52 females. The frequency of HBsAg was 61.6% in acute hepatitis, 69.2% in chronic hepatitis, 75.3% in cirrhosis, and 83.3% in hepatoma, with an average of 68.9% for all categories combined. Among 37 hepatoma cases, those under 40 years of age were 100% antigen positive, those 40-49 years of age were 88.9% positive, and those 50+ years of age were 70% positive, or an average of 86.5% for all ages combined.

(3) Family study: Among 19 mothers of hepatoma patients, 78.9% were positive for HBsAg, but only 25% of fathers were positive. Among asymptomatic HBsAg carriers, 34.7% of 49 mothers and 21.4% of fathers were antigen+.

(4) Death certificate analysis: The population of Taiwan in 1974 was approximately 16 million, of which 52.5% were males and 47.5% females. In 1974 there were 74,760 deaths, and at least 70,000 deaths in 1975 and 1976.

The 3 leading causes of death in Taiwan in 1973 were: 1st, cerebrovascular disease - 73.3 per 100,000; 2nd, malignant neoplasms - 62.3 per 100,000; and 3rd, heart disease - 53.4 per 100,000. Malignancies rank 3rd both in Taiwan and the U.S., when the age-adjusted rate is considered. Cirrhosis in Taiwan ranked 8th, with a rate of 15.6.

During the years 1969 through 1973 the 3 leading cancers in Taiwan were: 1st, liver cancer; 2nd, stomach cancer; and 3rd, lung cancer. From 1969 through

1973 approximately 1 out of every 5 cancer deaths was liver cancer. In 1973 13.1% of all deaths in Taiwan were from cancer, having increased from 11.4% in 1969. During 1968-1973 at least 74% of hepatoma deaths, and at least 72% of cirrhosis deaths occurred in males. Both hepatoma and cirrhosis rates show a linear arithmetic rise in relation to age. Chinese males have 5 to 15 times higher hepatoma rates than U.S. males. When hepatoma and cirrhosis mortalities are combined, the age-specific mortality curve in relation to HBsAg carriers forms an almost-straight line in both sexes.

The male death rates for hepatoma and cirrhosis by county and major city have been determined. The mean mortality in Taiwanese males from hepatoma 1970-1973 was 17.4 per 100,000, varying from 12.0 in Nantou county to 26.3 in the city of Chilung. The mean annual cirrhosis mortality in males 1970-1973 was 20.5, varying from 15.8 in Taipei county to 35.3 in Peng Hu county. Combining the mortality rates for hepatoma and cirrhosis yields 37.9 per 100,000, varying from 31.7 in Miaoli county to 60.8 in Hualien county.

Significance to Biomedical Research and the Program of the Institute. Thousands of persons die each year from hepatoma, cirrhosis, and chronic hepatitis. Recent studies have defined various populations which are at high risk to these diseases, and various modes of transmission. Other studies are being conducted to determine the efficacy of hepatitis B specific hyperimmune gamma globulin and hepatitis B vaccines in the prevention of infection. Such preventive measures will be of greater importance if it can be established that hepatoma and other chronic liver disease also result from HBV infection. Furthermore, if persons with HBsAg are at increased risk for developing chronic liver disease, increased research efforts will be justified in attempts to convert HBsAg carriers to non-carriers. The National Cancer Institute's Viral Oncology Program has been engaged in determining whether any viruses are oncogenic or predispose to oncogenicity, and the Environmental Epidemiology Branch of the Field Studies and Statistics Area is interested in the epidemiology of diseases caused by environmental agents. This particular study of HBV is thus closely related to the objectives of both these programs.

Proposed Course of Project. Only the Prospective Study was recommended for continued funding by NCI, since such a long-term follow-up has not been done elsewhere. Further support for the case-control study, family study, and death certificate analysis was not recommended. In the Prospective Study an additional 10,000 to 15,000 men will be studied and tested for HBsAg, anti-HBsAg, and α-fetoprotein. Negative controls will be selected for each HBsAg positive by taking the next negative person on the list from the same province who is = to, + or - 1 year of age of the cohort person. All deaths will be investigated by visiting families and reviewing clinical records. The aflatoxin studies have been deferred. Perhaps, t a later date, aflatoxin experts can investigate the matter. Perhaps a dietary history and an occupational history questionnaire can be developed in collaboration with the Environmental Epidemiology Branch, to be applied to subjects in the Prospective Study, and to others if feasible.

Date Contract Initiated: February 13, 1976.

Current Annual Level: $140,000.

ANNUAL REPORT

CARCINOGENESIS PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE

July 1, 1976 through September 30, 1977

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I. OVERVIEW

A. INTRODUCTION

The vast majority of cancers, affecting people throughout the world, appears to be caused by external chemical and physical agents. Due to the rapidly growing awareness of this fact, great emphasis has been placed on the identification and definition of such carcinogenic hazards and on the opportunities for preventing the development of cancers by interfering with the progression of events linking exposure to end results. Solid evidence has already been obtained on the role of a number of chemical and physical agents in the causation of human cancers, including, for example: (1) tobacco smoking which is strongly associated with cancers of the lung, larynx, oral cavity, esophagus, bladder, and pancreas; (2) ultraviolet light and certain chemical exposures in the causation of cancers of the skin; (3) ionizing radiation in the causation of leukemias, cancers of the lung, thyroid, breast, skin, and bone; and (4) consumption of alcoholic beverages associated with cancers of the larynx, esophagus, and other neoplasms. In addition, host factors have been defined for several population groups which have an effect on their susceptibility.

Much of the knowledge already acquired on cancer pathogenesis could be applied at this time to prevent the development of cancers in the population. Among these actions might be, for example, cessation of smoking, reduction of alcohol consumption, adoption of a "prudent" diet, protection from excessive exposure to ultraviolet light, and protection from occupational exposure to carcinogens. On the other hand, societal decisions, frequently beyond individual control, are involved in the implementation of preventive measures requiring changes in technology, development, and adoption of less hazardous products, and regulatory control of exposures. In striving for such change and objective, a well-documented body of information is needed. It is one of the responsibilities of a carcinogenesis program within NIH to provide such a basis of scientific knowledge and documentation.

A major societal decision is also involved in establishing priorities for developing and supporting the basic and applied research needed to provide a more extensive knowledge and documentation on the substances and exposures that cause cancers, on the factors which determine our susceptibility to them, and finally, on the biologic and chemical mechanisms that can be "exploited" to prevent, inhibit, or delay the onset of cancers in the population.

Protecting the population from chemical and physical carcinogenic hazards and their effects has been clearly recognized as an urgent and essential task in our society, and a high national priority for this mission has been forcefully indicated by the Cancer Act, by Congress, and by the Administration. The Carcinogenesis Program continues to be developed to serve towards the accomplishment of these national goals.

The field of environmental carcinogenesis has received new emphasis by the National Cancer Advisory Board, which has established a Subcommittee on Environmental Carcinogenesis. Staff from the Carcinogenesis Program has

worked closely with this new Subcommittee.

The extent to which research on chemical and physical carcinogenesis can be appropriately defined as "environmental carcinogenesis" is hard to assess. The NCI's commitment to a national program on chemical and physical carcinogenesis and cancer prevention clearly extends beyond what can be specifically classified as its environmental aspects. Yet these extend so widely that most of the implications of carcinogenesis studies, even at the level of molecular mechanism studies, are definitely relevant to the assessment of environmental cancer hazards for man and to their prevention.

A survey of current activities in the Carcinogenesis Program using a strict definition, has indicated that about 60-70% of its contract support is devoted to research on environmental chemical and physical factors, either by bioassay or for studies directly aimed at defining the role of these factors in carcinogenesis. However, much of the remaining 30-40% of program support is addressed to the development of better biological models and to mechanism studies which will provide a necessary basis for the future understanding of carcinogenic effects and for the development of preventive measures.

The study of the chemical and physical causes of cancer has always been intimately related to their environmental occurrence. Environmental carcinogenesis thus appears to be an integral and inseparable part of the field of chemical/physical carcinogenesis.

The effectiveness of the Program is determined not only by the level of financial support provided to it, but above all by the availability of a staff of high scientific competence, fully dedicated to its imposing task, and by the collaboration of a large, outstanding scientific community in universities and research institutes as well as in industrial and commercial laboratories.

The Program has developed and extensively used a planning and review system for collaborative research, which provides the invaluable help of a highly qualified group of scientific leaders and advisors from outside institutions for the critical development of our activities and their peer review. Much of the success of the Program is due to the collaborative constructive spirit with which scientists in over 150 institutions across the country and abroad have joined forces in this demanding fight against the induction of cancer.

B. PROGRAM PLANS

The National Cancer Institute's Carcinogenesis Program originated as the Carcinogenesis Studies Branch, established in 1961 as part of the Field Studies Area. In 1966 the Carcinogenesis Program was organized under the Office of the Associate Scientific Director for Carcinogenesis within the newly established Etiology Area. In 1968 new intramural research branches were established and a major contract-supported collaborative Program was developed. In 1972 the Etiology Area became the present Division of Cancer Cause and Prevention. The Tobacco Research portion of the Carcinogenesis Program was transferred to the Division in 1973 and expanded as a separate Smoking and Health Program.

The Carcinogenesis Program has developed into a complex effort based on the collaboration of some 200 laboratories in different institutions including the intramural research laboratories, all devoted to the study of carcinogenesis and cancer prevention, all committed to a collaborative approach to the field. Carcinogenesis research has developed into a complex multidisciplinary approach where investigators of many different disciplines work closely together--human and experimental pathologists, chemists, toxicologists, epidemiologists, occupational health specialists, biochemists, enzymologists, immunologists, cell biologists, molecular biologists, geneticists, etc. from government, universities, and research institutes as well as from industrial and commercial laboratories. The Program is characterized by the integration of basic research and targeted research applied toward specific problems.

Late in the 1976 fiscal year a decision was made at the Institute level that the Carcinogenesis Program would be divided into two functional and organizational units: The Carcinogenesis Research Program and the Carcinogenesis Testing Program; the former as a research and development effort, the latter to give organizational and operational identity to the Bioassay Testing Program and the information resource activities of the Division.

The establishment of the Carcinogenesis Testing Program as a major program area within the Division of Cancer Cause and Prevention would constitute the fourth program organization within this division and would provide for it equal operational and organizational status with the three existing programs: Carcinogenesis Research, Field Studies and Statistics, and Viral Oncology.

It is proposed that the Carcinogenesis Testing Program would have three Intramural Branches: Technical Information and Resources Branch, Tumor Pathology Branch, and Toxicology Branch. In addition, a collaborative, contract-supported program for in vitro and in vivo bioassay testing would be maintained.

The Carcinogenesis Testing Program will be concerned with the direction, coordination, and evaluation of an applied research program on chemical and physical causes of cancer and their prevention. The program will specifically administer carcinogen testing procedures and related animal model bioassay methods through intramural laboratories and contracts. It will develop and standardize models for carcinogenesis testing and develop and maintain

information resources and data analysis activities related to environmental carcinogenesis. This program will advise the Director of the Division on carcinogenesis testing and will support the activities of the National Cancer Advisory Board and other scientific advisory committees with regard to the program. This program will have major responsibility for interaction and coordination with the Clearinghouse for Environmental Carcinogenesis through the Office of the Division Director.

The creation of a division-level program of this magnitude requires reorganization approval from the Department of Health Education and Welfare through the National Cancer Institute, the National Institutes of Health, and the Public Health Service. Final approval through these channels was still pending three months prior to the close of FY 77.

Since the formalization of the program reorganization plans is not yet complete, this annual report has been organized in a manner to best reflect the functional operation of the program as it now exists. Nevertheless, a fully coherent presentation of the current program defies description at a time when position vacancies are being filled and responsibilities and program plans are in a constant state of transition.

The total Carcinogenesis Program of the Division as reflected in this report (see 2. Organizational Structure and Management) is under the direction of the Acting Associate Director for Carcinogenesis, Dr. Gio B. Gori. The six intramural branches and the Office of the Coordinator for Collaborative Research (Acting Chief, Dr. Thomas B. Owen) are articulated through this office. All of the Collaborative Research Program for Carcinogenesis is currently managed and administered through four Operational Units: Bioassay Operational Unit, Biology Operational Unit, Chemistry Operational Unit, and Interdisciplinary Programs and Resources Unit. Each Operational Unit provides program management and administration for a number of Segment activities. The responsibilities of the Segments remains essentially unchanged at this time from previous reports. Segments continue to have responsibility for developing specific programs based on their fundamental objectives within the framework of the overall Carcinogenesis Program.

C. MANAGEMENT AND REVIEW SYSTEM

The complexity of disciplines and scientific trends in contemporary carcinogenesis research indicates that many competent voices should be allowed to participate in the structuring of priorities at the national level. Toward such end, a new management and review structure has been proposed for the Carcinogenesis Research Program. It is desirable that advice and direction be received from all segments of the research community. The mechanisms for such input are numerous, including scientific workshops to develop program goals and consultant recommendations on specific research projects. A public advisory committee, the Carcinogenesis Scientific Advisory Committee (CSAC), will serve to solicit and channel the recommendations received from the scientific community.

This eight-member committee has been chartered (for current membership list, see Section 2(c)) and its first meeting was held March 25, 1977. It will provide (1) advice on overall program needs and priorities, both intramural and collaborative, (2) scientific review of intramural research activities, and (3) selection and scientific basis for major areas of program development. This advisory committee will provide a resource for planning and initiating research on a program-wide basis, as contrasted to the more narrowly Segment-oriented research activities.

The Associate Director for the Carcinogenesis Program and his staff are responsible for the overall conduct of the operations and decision making. They will be assisted by the CSAC through the following cyclical process:

A. The CSAC will define the most important and promising areas of carcinogenesis research.

B. The program director and staff will convene workshops or Program Development Consultations (PDC) on the topics identified by the CSAC, where scientific peer experts may discuss the opportunities for specific research projects and alternatives.

C. The CSAC will consider the projects suggested by the workshops within the overall needs of a national carcinogenesis program and will recommend priorities for the execution of these projects.

D. The program director and staff will relate these recommendations to NCI budget realities and will coordinate with other agencies and programs in carcinogenesis research to avoid duplication and promote cross-fertilization of ideas; then those projects which can be funded by research contracts or by cancer research emphasis grants (CREGS) will be advertised nationally.

E. The contract proposals and CREG applications received will be submitted by the Program staff to scientific merit review by standing committees of peers from the scientific community. Approved projects will be awarded by the Program staff and project officers will be assigned.

F. New data generated by the Program, or by other research worldwide, will be monitored, submitted to, and reviewed by the CSAC and workshop attendees, to eventually modify existing priorities and to advance established understanding in carcinogenesis research.

The dynamics of cancer research suggest that this review cycle could be renewed annually.

The Collaborative Carcinogenesis Research Program is organized to offer a targeted parallel alternative to unstructured grant-supported research, toward the definition of methods for the ultimate control of cancer. As such, the Program strives to balance the allocation of priorities and resources according to an assessment of national needs. The necessary choices are determined by a mix of objective information, such as epidemiological realities and established scientific understanding, and of creative invention from the community of scientists.

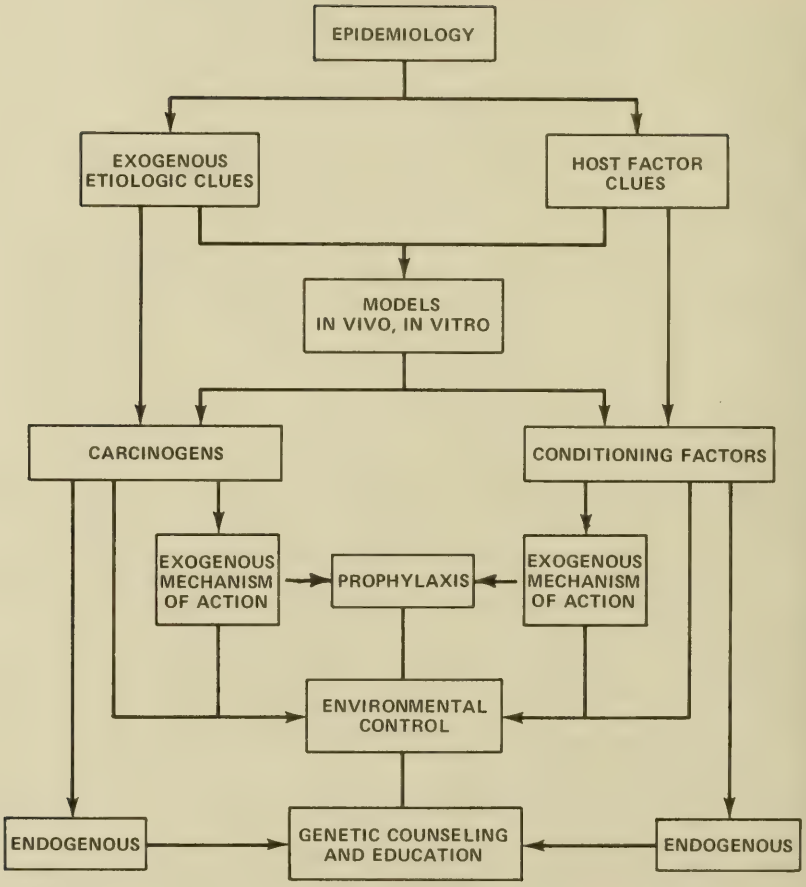
An overall mainline logic flow (Figure 1) will utilize epidemiological clues of primary carcinogens and of those host and environmental factors which influence host resistance or susceptibility. Epidemiologic and scientific knowledge would then help in selecting successful animal, man, or in vitro experimental models. In turn, investigations with these models would clarify the nature and mode of action of carcinogens and modifying factors and would suggest methods for their elimination or control.

The program, in collaboration with the CSAC, the Scientific Technical Review Committees A and B, and other eminent scientists, has been developing an environmental carcinogenesis classification scheme to be used as a guide in the classification of individual research projects, both ongoing and proposed. In this classification scheme, carcinogenesis research projects are classified according to a principal descriptor and some secondary descriptors, reflecting the stated scope of the project, its primary and secondary objectives, and the interest of the investigator. Thus, using the scheme as a guide to determine current funding patterns, the Carcinogenesis Research Program, in its assessment of national needs, will hope to achieve a balance of programs and avoid duplication of effort in assigning priorities to research areas.

Topical workshops suggested by the CSAC will be selected and their resulting recommendations framed in the perspective of this classification scheme. The workshops or PDCs will be attended by a small group of specialists meeting for one or two days. Attendees will receive considerable input from the Program staff and enough introduction to insure productivity during the meeting. Twenty to thirty workshops are anticipated every year, allowing participation of two hundred to three hundred scientists. This process will generate research ideas from a cross-section of scientific expertise and opinion and, hopefully, may optimize the probability of advancing priority knowledge in carcinogenesis. It will also produce a backlog of desirable research projects to support requests for additional resources and funds, and to provide a realistic choice of top project priorities within the carcinogenesis program.

The CSAC will provide review of new and ongoing projects for priority, need, and relevance to the goals of this national (and truly international) program. The Carcinogenesis Program Scientific Review Committees A and B, on the other hand, will continue to provide a peer review of research proposals submitted to the Program in order to determine the scientific merit of the research proposed and the technical competence of the proposed staff. Each committee, chartered as a Public Advisory Group, maintains a membership of ten extramural, peer experts who are knowledgeable in the fields necessary to review the total Carcinogenesis Research and Testing Program's proposals. Committees A and B met a total of nine times in FY 77 and reviewed approximately 210 proposals.

As in the past, there will be a continued heavy involvement of intramural staff in the role of project officers. However, the National Cancer Institute project officers cannot be expected to encompass all the scientific competence necessary to monitor each project; they will be assisted in their task by expert consultants from the scientific community.



CARCINOGENESIS RESEARCH PROGRAM MAINLINE LOGIC FLOW

FIGURE 1:

The intramural research component of Carcinogenesis Research will function independently of the extramural or collaborative program staff. Intramural scientists will conduct their own research and will participate in the structuring of the National Carcinogenesis Program much as outside scientists do; for instance, they may be attending workshops where their individual expertise is relevant and will also serve as NCI project officers where their interest applies.

The responsibilities of the major organizational components will be as follows:

Associate Director (AD) - The Associate Director will have overall responsibility for all aspects of the Program, including definition of research direction, relevance of subprograms operation, and project review. He will interpret program aims and activities to the National Cancer Advisory Board and the Carcinogenesis Program Advisory Committee. The director will provide operating guidelines to the program staff.

Coordinator for Collaborative Research (CCR) - Implementation of operation guidelines received from the AD will be the responsibility of the CCR. He will work closely with the AD and Advisory Committees to integrate Program activities. The CCR will provide budgetary plans and restraints in assessing priority needs for the Program. Responsibility for submitting individual project plans to the Technical Review Committee and for appropriate selection of Program and project consultants lies with the CCR. Coordinating activities and priorities among the four major research areas will be a prime objective of this organizational component.

Program Segment Director (PSD) - The Carcinogenesis Research Program will have three main investigative segments: cancer models and organ sites, molecular and metabolic carcinogenesis, and conditioning and modifying factors. A PSD would be appointed for each segment of research. It will be the responsibility of the PSD to develop new Program concepts, design research projects, prepare RFPs and CREGs as necessary, and to engage in effective intercommunication at all levels of Program operation. The PSD will provide the necessary informational input to the CSAC and execute its directions for program inception.

Carcinogenesis Program Management Group (CPMG) - This group will be composed of the Associate Director, Coordinator for Collaborative Research, and an Administrative Officer. They will function as a central staff review for the Carcinogenesis Program, authorizing program actions and budgets, recommending program and staff activities, and providing an interface among the staff, the advisory committees, and the Division of Cancer Cause and Prevention. The CPMG will also allocate funds for each defined sub-program based upon the recommendations of the CSAC and the resources allocated to the Program from general NCI funding.

The following operating procedures will be used within the organizational scheme for processing New, Renewal, and Unsolicited Proposals:

New Projects (Figure 2) - New project ideas will come primarily from the CSAC, CPMG, program staff, consultants, and workshops. A project plan will be prepared and submitted to the CSAC for a review of Program relevance. If the

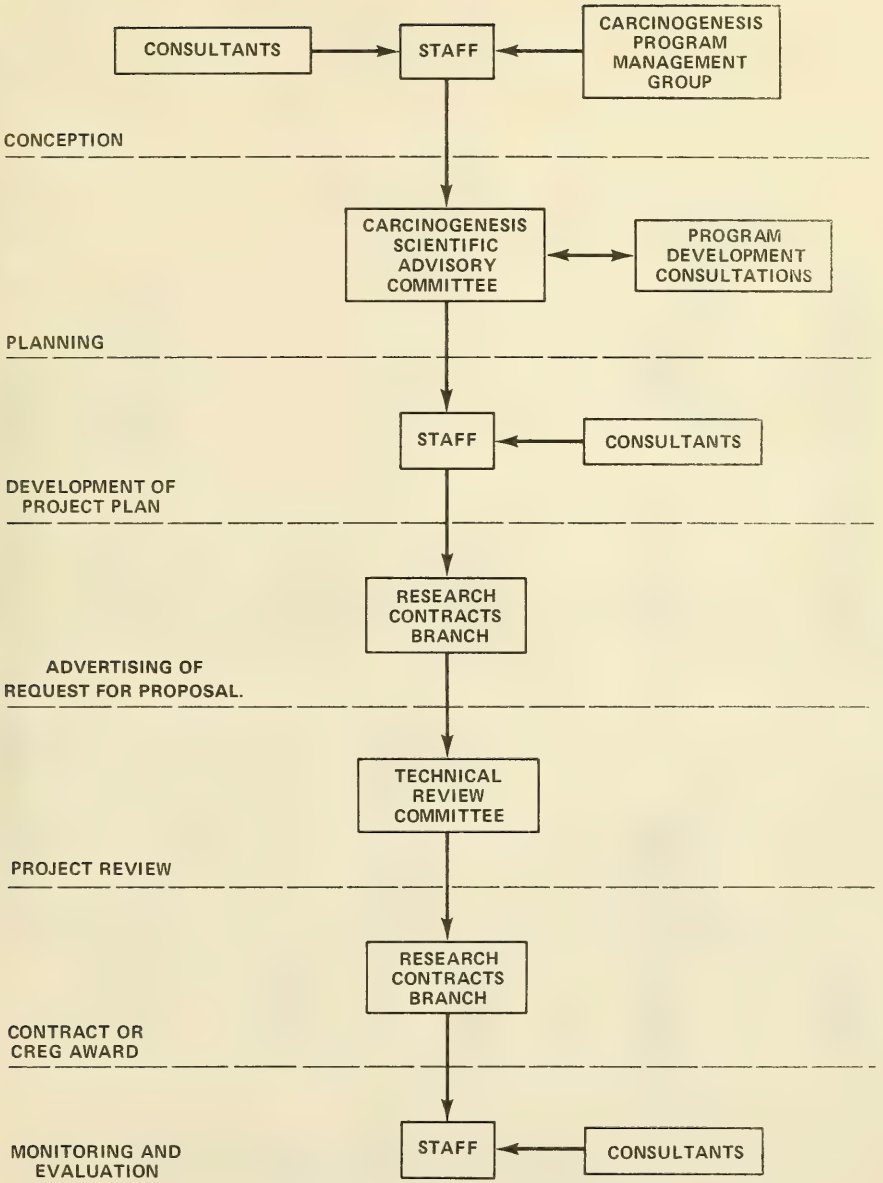
project plan is approved, a request for proposal will be prepared by the Program staff. The research contracting branch will determine the proper contractive form (contract or CREG), advertise the RFP/RFA, and accept proposals. These will be evaluated by the technical review committee and ad hoc consultants. Based on the committee's recommendation, a contract or CREG will be negotiated and awarded.

Renewed Projects (Figure 3) - The procedures for renewing projects will be basically the same as initiating a new proposal, except that no new project plan will be required. Project status will be reviewed by the CPMG and a decision made concerning renewal based on program priorities established by the CSAC. A technical evaluation will be made of the renewal proposal. Based on the review committee recommendations, a renewal contract will be negotiated and awarded.

Unsolicited Proposals (Figure 4) - Unsolicited proposals will be reviewed by the program staff for relevance and submitted to the CSAC. If approved, it will then be reviewed by the technical review committee. Based on the review recommendations, a decision will be made to negotiate and award a research contract.

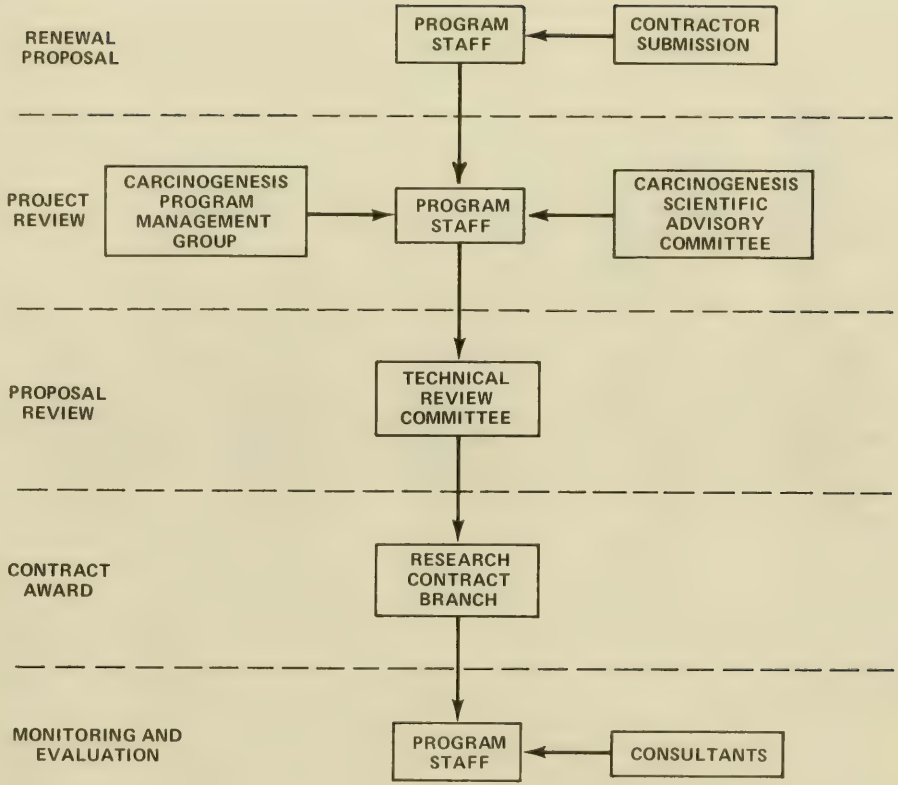
As always, major requirements of time and effort have gone into the daily operational management involved in planning and obtaining approval of new programs, development of project plans, development and issuance of requests for proposals, proposal review, contract monitoring, and meeting ever-increasing requirements for documentation under the Freedom of Information Act and the Privacy Act. The major management challenge which remains is to develop and maintain a scientifically sound program in a broad multidisciplinary field with very limited staff resources.

The staff has met this challenge but it is increasing its serious over-extension to a point which raises the question of how long the present pace can be maintained. Recognition is due the Carcinogenesis Program staff to whose extraordinary, invaluable, and untiring efforts this program owes its development. In spite of the continued imposition of strict limitations in personnel positions, the Program has maintained an excellent, highly productive intramural research activity together with a rapidly expanding collaborative program; both were made possible by the highly dedicated, often strenuous commitment of the members of the staff.

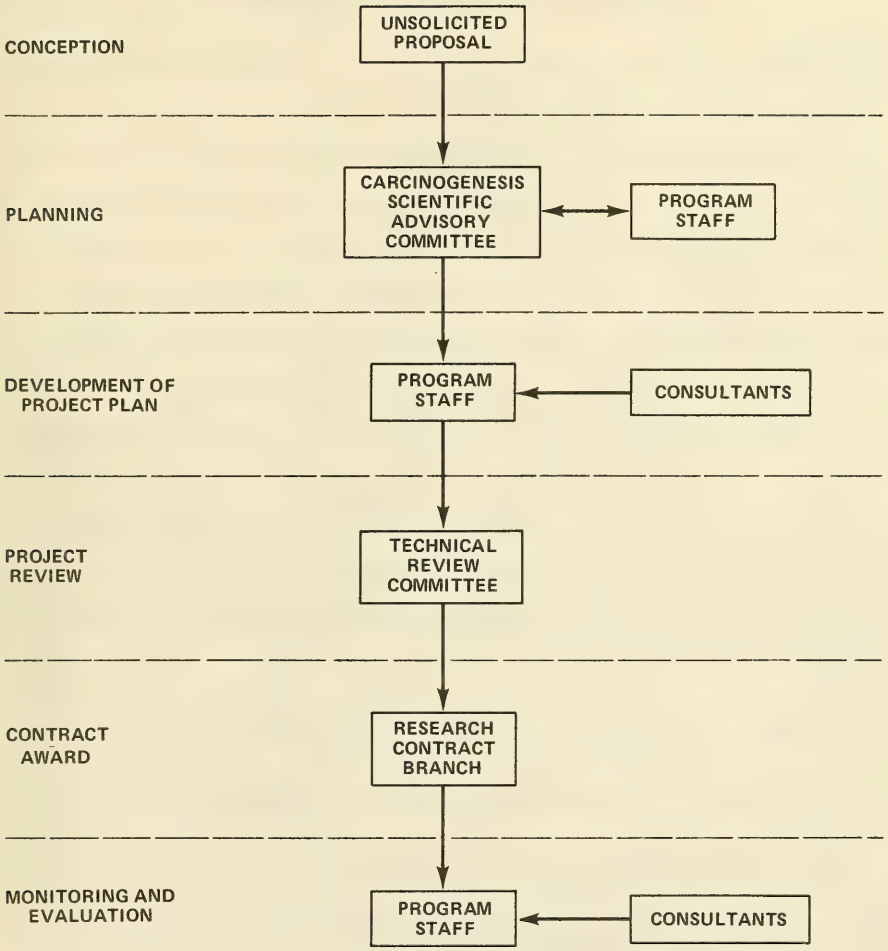


REVIEW PROCEDURES FOR NEW PROJECTS

FIGURE 2:



REVIEW PROCEDURES FOR PROJECT RENEWAL
FIGURE 3:



REVIEW PROCEDURES FOR UNSOLICITED PROPOSAL

FIGURE 4:

2. ORGANIZATIONAL STRUCTURE AND MANAGEMENT

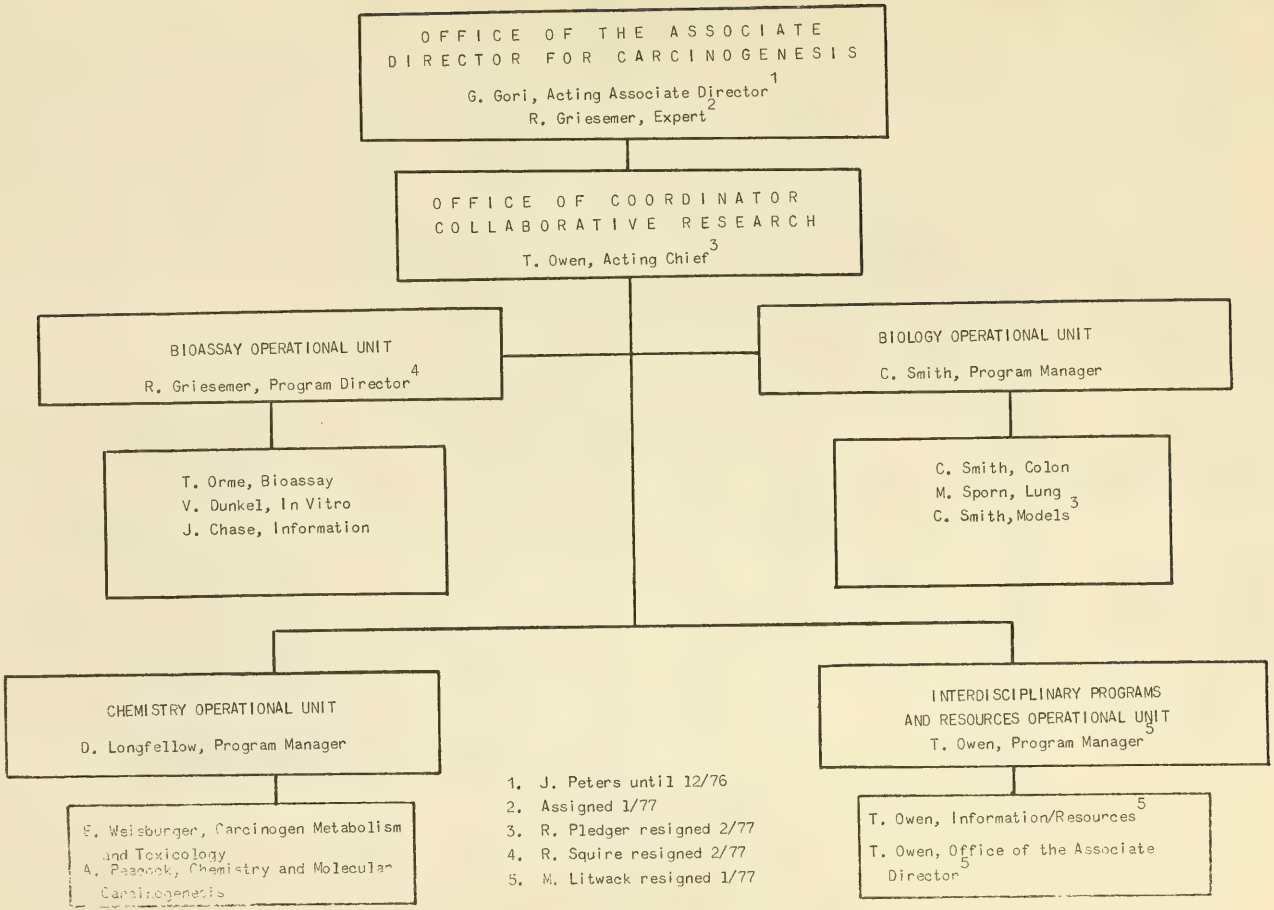
The organization of the Carcinogenesis Program is presently divided into two major components: intramural program and collaborative program. The former is articulated in the Office of the Associate Director and in six branches, each with several sections and units. The latter is articulated into four operational units comprised of a total of ten segments. Each operational unit is directed by a scientific program manager. Complementing segments, which together comprise an operational unit, are each directed by a senior staff member; other members of the intramural staff serve as project officers on individual contracts. Segments are assisted by individual ad hoc advisors from outside NCI. Two technical peer review committees provide expert review of specific project plans and proposals submitted to the program. They provide recommendations concerning the scientific merit of the research and technical competence of the proposed staff prior to the award of contracts or other appropriate support instruments.

This section is divided into the following three units:

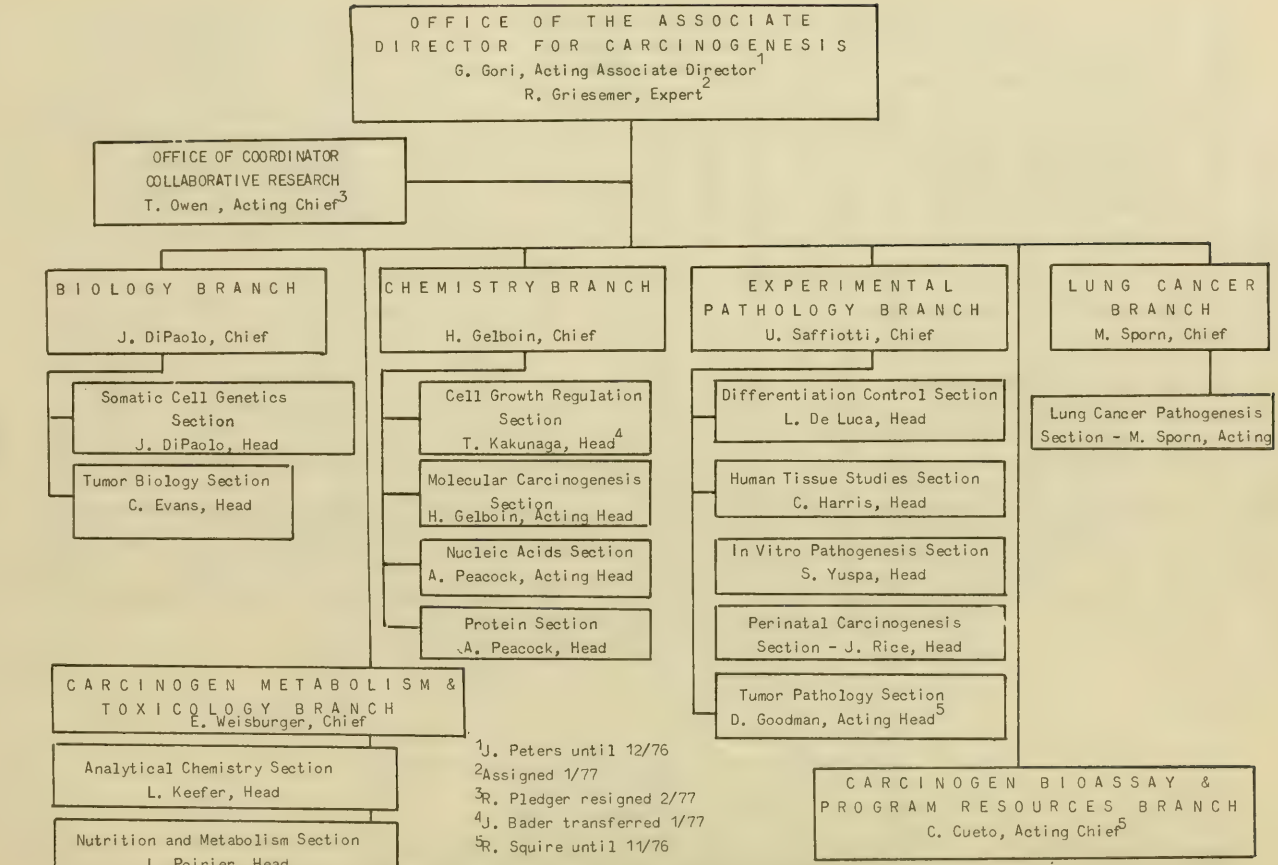
A. Diagram of the Collaborative Research Organization

B. Diagram of the Intramural Organization

C. Listing of Advisory Groups and Consultants within the Carcinogenesis Program


OFFICE OF THE ASSOCIATE DIRECTOR FOR CARCINOGENESIS
G. Gori, Acting Associate Director 1
R. Griesemer, Expert 2
OFFICE OF COORDINATOR COLLABORATIVE RESEARCH
T. Owen, Acting Chief 3
BIOASSAY OPERATIONAL UNIT
R. Griesemer, Program Director 4
T. Orme, Bioassay
V. Dunkel, In Vitro
J. Chase, Information
BIOLOGY OPERATIONAL UNIT
C. Smith, Program Manager
C. Smith, Colon
M. Sporn, Lung
C. Smith, Models 3
CHEMISTRY OPERATIONAL UNIT
D. Longfellow, Program Manager
E. Weisburger, Carcinogen Metabolism and Toxicology
A. Peacock, Chemistry and Molecular Carcinogenesis
INTERDISCIPLINARY PROGRAMS AND RESOURCES OPERATIONAL UNIT
T. Owen, Program Manager 5
T. Owen, Information/Resources 5
T. Owen, Office of the Associate Director 5
1. J. Peters until 12/76
2. Assigned 1/77
3. R. Pledger resigned 2/77
4. R. Squire resigned 2/77
5. M. Litwack resigned 1/77

A. COLLABORATIVE RESEARCH ORGANIZATION



[1] J. Peters until 12/76
[2] Assigned 1/77
[3] R. Pledger resigned 2/77
[4] J. Bader transferred 1/77
[5] R. Squire until 11/76

B. INTRAMURAL ORGANIZATION

C. ADVISORY GROUPS AND CONSULTANTS

(1) The Carcinogen Bioassay Staff Group provides a coordinating and planning function to the Bioassay Program at the senior staff level. Membership of this group is as follows:

Dr. Richard A. Griesemer, Acting Director, Bioassay Program, *Chairman*
Ms. Joan W. Chase, Manager, Information
Dr. Kenneth C. Chu, Carcinogen Bioassay and Program Resources Branch
Dr. Cipriano Cueto, Jr., Acting Chief, Carcinogen Bioassay and Program Resources Branch
Dr. J. Fielding Douglas, Expert Consultant, Carcinogen Bioassay and Program Resources Branch
Dr. Virginia C. Dunkel, Manager, In Vitro
Dr. Dawn G. Goodman, Acting Head, Tumor Pathology Section, Experimental Pathology Branch
Dr. Thomas W. Orme, Manager, Bioassay
Dr. Sidney Siegel, Carcinogen Bioassay and Program Resources Branch
Dr. Jerrold M. Ward, Tumor Pathology Section, Experimental Pathology Branch
Mr. Damian I. Crane, Administrative Officer, Carcinogenesis Program
Mr. Thomas Hooven, Administrative Assistant, Carcinogenesis Program
Ms. Melva Weber, Expert, Office of Cancer Communications, NCI

(2) The Carcinogenesis Bioassay Program Review Group is a committee of staff scientists who provide review of proposed activities for priority and relevance within the Carcinogenesis Bioassay Program. Membership of this groups consists of representatives of all the major elements in the Program.

Dr. Richard A. Griesemer, Acting Director, Bioassay Program, *Chairman*
Ms. Joan W. Chase, Manager, Information, *Executive Secretary*
Dr. Thomas P. Cameron, Assistant Coordinator for Environmental Cancer
Dr. Cipriano Cueto, Jr., Acting Chief, Carcinogen Bioassay and Program Resources Branch
Dr. J. Fielding Douglas, Expert Consultant, Carcinogen Bioassay and Program Resources Branch
Dr. Virginia C. Dunkel, Manager, In Vitro
Dr. Dawn G. Goodman, Acting Head, Tumor Pathology Section, Experimental Pathology Branch
Dr. Thomas W. Orme, Manager, Bioassay
Dr. Elizabeth K. Weisburger, Chief, Carcinogen Metabolism and Toxicology Branch

(3) The Carcinogenesis Intramural Program Group provides a coordinating and planning function to the intramural program at the senior staff level. Since the vast majority of the intramural professional staff is now active in the planning and monitoring of the overall program, this group provides a vital interface between intramural and collaborative research programs. Membership of this group is limited to the staff of the Office of the Associate Director, the Administrative Officer, and the five branch chiefs.

[1]Dr. Gio B. Gori, Acting Associate Director, *Chairman*
Mr. Damian I. Crane, Administrative Officer
Dr. Joseph A. DiPaolo, Chief, Biology Branch
Dr. Harry V. Gelboin, Chief, Chemistry Branch
[2]Dr. Thomas B. Owen, Acting Chief, Office of the Coordinator for Collaborative Research
Dr. Umberto Saffiotti, Chief, Experimental Pathology Branch
Dr. Michael B. Sporn, Chief, Lung Cancer Branch
Dr. Elizabeth K. Weisburger, Chief, Carcinogen Metabolism and Toxicology Branch

(4) The Carcinogenesis Program Management Group is a committee of staff scientists who provide review of proposed activities for priority and relevance within the Carcinogenesis Program. Membership of this group consists of representatives of all the major elements in the Program: the Office of the Associate Director, the branch chiefs from the intramural scientific staff, segment directors from collaborative program scientific staff, as well as a representative from the Division of Cancer Research Resources and Centers.

[1]Dr. Gio B. Gori, *Acting Chairman*
Ms. A. Christine Manuel, *Acting Executive Secretary*
Dr. Joseph A. DiPaolo, Chief, Biology Branch
Dr. Thaddeus Domanski, Program Director for Carcinogenesis, Division of Cancer Research Resources and Centers, NCI
Dr. Harry V. Gelboin, Chief, Chemistry Branch
[2]Dr. Thomas B. Owen, Acting Chief, Office of the Coordinator for Collaborative Research
Dr. Andrew C. Peacock, Director, Chemistry and Molecular Carcinogenesis Segment
Dr. Umberto Saffiotti, Chief, Experimental Pathology Branch
Dr. Michael B. Sporn, Director, Lung Cancer Segment
Dr. Elizabeth K. Weisburger, Director, Carcinogen Metabolism and Toxicology Segment

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[1]Dr. James A. Peters until December, 1976
[2]Dr. Richard A. Pledger resigned February, 1977

(5) The Carcinogenesis Program Scientific Advisory Committee provides advice on matters of scientific program policy relative to the intramural and collaborative research programs of the Carcinogenesis Program in the Division of Cancer Cause and Prevention. The Committee was established on January 8, 1976. The first formal meeting was held March 25, 1977 in Bethesda, Maryland.

Dr. Louis Siminovitch, University of Toronto, Toronto, Ontario, Canada, *Chairman*
Dr. Gio B. Gori, *Executive Secretary*
Dr. Roswell K. Boutwell, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
Dr. Fredric J. Burns, New York University Medical Center, Institute of Environmental Medicine, New York, New York
Dr. Ercole Cavalieri, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska
Dr. R. J. Michael Fry, Argonne National Laboratory, Argonne, Illinois
Dr. Margaret Howell, 3883 Turtle Creek Drive, Dallas, Texas
Dr. Jeffery Ross, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
Dr. David C. Ward, Yale University School of Medicine, New Haven, Connecticut

(6) The Carcinogenesis Program Scientific Review Committees A and B provide expert peer review of proposals submitted to the Program. Based on an evaluation of the scientific merit of the proposed work and on an evaluation of the technical competence of the proposed staff, the Committees provide recommendations concerning the award of contracts or other appropriate support instruments.

Carcinogenesis Program Scientific Review Committee A

Dr. Robert E. Greenfield, National Bladder Cancer Project, St. Vincent Hospital, Worcester, Massachusetts, *Chairman*
[2]Dr. Carl E. Smith, *Executive Secretary*
Dr. Howard A. Bern, Cancer Research Laboratory, University of California, Berkeley, California
Dr. Louis M. Fink, University of Colorado Medical School, Denver, Colorado
Dr. M. Edward Kaighn, Pasadena Foundation for Medical Research, Pasadena, California
Dr. Louise S. Lombard, Argonne National Laboratory, Argonne, Illinois
Dr. Robert H. Palmer, The Rockefeller University, New York, New York
[3]Dr. Roy Ritts, Mayo Graduate School of Medicine, Rochester, Minnesota
Dr. Evelyn M. Rivera, Michigan State University, East Lansing, Michigan
Dr. Andrew Sivak, Arthur D. Little, Inc., Cambridge, Massachusetts

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[2]Dr. Richard A. Pledger resigned February, 1977
[3]Transferred from Committee B November, 1976

Carcinogenesis Program Scientific Review Committee B

Dr. Floie M. Vane, Hoffmann-LaRoche, Inc., Nutley, New Jersey, *Chairman*
[4]Dr. David G. Longfellow, *Executive Secretary*
Dr. Gerald L. Bartlett, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania
Dr. Phillip Issenberg, Eppley Institute for Research in Cancer, University of Nebraska, Omaha, Nebraska
Dr. Adele Kostellow, Albert Einstein College of Medicine, Bronx, New York
Dr. Prabhakar D. Lotlikar, Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania
Dr. Veronica M. Maher, Michigan State University, East Lansing, Michigan
Dr. Danuta Malejka-Giganti, Laboratory for Cancer Research, Veterans Administration Hospital, Minneapolis, Minnesota
Dr. Paul Talalay, The Johns Hopkins University School of Medicine, Baltimore, Maryland
Dr. Nien-chu C. Yang, University of Chicago, Chicago, Illinois

(7) The Chemical Selection Working Group, Experimental Design Group, and Data Evaluation Group are basic working groups of the Carcinogenesis Bioassay Program. NCI membership is as follows:

Chemical Selection Working Group

Dr. Herman F. Kraybill, *Chairman*
Dr. Richard A. Griesemer, *Adviser*
Dr. Thomas P. Cameron
Dr. J. Fielding Douglas
Dr. Virginia C. Dunkel
Dr. Lionel Poirier
Dr. Sidney Siegel
Dr. Elizabeth K. Weisburger

Experimental Design Group

Dr. Cipriano Cueto, Jr., *Chairman*
Dr. Thomas P. Cameron, *Adviser*
Dr. Richard A. Griesemer, *Adviser*
Dr. Thomas W. Orme, *Adviser*
Dr. Kenneth Chu
Dr. J. Fielding Douglas
Dr. Dawn G. Goodman
Dr. Elizabeth K. Weisburger

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[4]Dr. Marcia D. Litwack resigned January, 1977

Data Evaluation Group

Dr. Cipriano Cueto, Jr., Chairman
Dr. Richard A. Griesemer, Advisor
Dr. Kenneth C. Chu
Dr. Dawn G. Goodman
Dr. Thomas W. Orme
Dr. Jerrold M. Ward

Close liaison has been established with the National Cancer Advisory Board and its Subcommittee on Environmental Carcinogenesis.

Other consultants who have been utilized by the Program throughout this fiscal year as contract site visitors, reviewers, individual technical advisors, or lecturers are as follows:

Dr. Joseph Arcos, Tulane University School of Medicine, New Orleans, Louisiana
Dr. Allan Balmain, The Beatson Institute for Cancer Research, Glasgow, Scotland
Dr. J. Carl Barrett, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Helmut Bartsch, International Agency for Research on Cancer, Lyon, France
Dr. Earl P. Benditt, University of Washington, Seattle, Washington
Dr. William F. Benedict, Children's Hospital of Los Angeles, Los Angeles, California
Dr. Judith Berliner, University of California at Los Angeles, Los Angeles, California
Dr. John Scott Bertram, Roswell Park Memorial Institute, Buffalo, New York
Dr. Wolfgang Blechschmidt, BASF Aktiengesellschaft, Ludwigshafen, Germany
Dr. Larry Bockstahler, Food and Drug Administration, Washington, D.C.
Dr. Jacques Bonnel, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Roswell K. Boutwell, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
Dr. Peter Brookes, Chester Beatty Research Institute, Buckinghamshire, England
Dr. Stuart Brown, Veterans Administration Hospital, Washington, D.C.
Dr. Miriam M. Brysk, University of Michigan, Ann Arbor, Michigan
Dr. George Buchi, Massachusetts Institute of Technology, Cambridge, Massachusetts
Dr. Harry Burchfield, New Iberia, Louisiana
Dr. Melvin Calvin, University of California, Berkeley, California
Dr. Elroy Cantrell, North Texas State University, Denton, Texas
Dr. William Caspary, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Bruce C. Casto, Biolabs, Inc., Northbrook, Illinois
Dr. Peter A. Cerutti, University of Florida, Gainesville, Florida
Dr. Jarvis T. Chan, Baylor College of Medicine, Houston, Texas
Dr. Ralph E. Christoffersen, University of Kansas, Lawrence, Kansas
Dr. Ernest Chu, University of Michigan Medical School, Ann Arbor, Michigan

Dr. Donald Clive, Burroughs Wellcome & Company, Research Triangle Park, North Carolina
Dr. Robert M. Coates, University of Illinois, Urbana, Illinois
Dr. Minor J. Coon, University of Michigan, Ann Arbor, Michigan
Dr. Herbert L. Cooper, Laboratory of Pathophysiology, DCBD, NCI, NIH
Dr. Richard Cramer, Philadelphia, Pennsylvania
Dr. Brian Crawford, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Lon Crosby, Enviro-Control, Rockville, Maryland
Dr. S. J. Danishefsky, University of California, Irvine, California
Dr. Raymond Daudel, Centre National de La Recherche Scientifique, Paris, France
Dr. Hector DeLuca, University of Wisconsin, Madison, Wisconsin
Dr. Frederick J. DeSerres, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
Dr. Thelma B. Dunn, Charlottesville, Virginia
Mr. James F. Engel, Midwest Research Institute, Kansas City, Missouri
Dr. Ronald Estabrook, University of Texas, Dallas, Texas
Dr. George M. Farrow, Mayo Clinic, Rochester, Minnesota
Dr. Robert S. Fontana, Mayo Clinic, Rochester, Minnesota
Dr. James R. Fouts, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
Dr. Gilbert H. Friedell, St. Vincent Hospital, Worcester, Massachusetts
Dr. N. E. Fusenig, Deutsches Krebsforschungszentrum, Heidelberg, Germany
Dr. Robert J. Gander, Johnson & Johnson, New Brunswick, New Jersey
Dr. James German, New York Blood Center, New York, New York
Dr. James R. Gillette, NHLBI, NIH, Bethesda, Maryland
Dr. DeWitt S. Goodman, Columbia University College of Physicians and Surgeons, New York, New York
Dr. John T. Grayhack, Northwestern University Medical School, Chicago, Illinois
Dr. Sidney Green, Food and Drug Administration, Washington, D.C.
Dr. Joe W. Grisham, University of North Carolina, Chapel Hill, North Carolina
Dr. Lawrence Grossman, Johns Hopkins University, Baltimore, Maryland
Dr. Phillip Grover, Chester Beatty Research Institute, London, England
Dr. C. L. Grubbs, IIT Research Institute, Chicago, Illinois
Dr. Dezider Grunberger, Columbia University, New York, New York
Dr. Michael R. Guerin, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Mr. William Haenszel, Illinois Cancer Council, Chicago, Illinois
Dr. Philip Hanawalt, Stanford University, Palo Alto, California
Dr. Ronald Harvey, University of Chicago, Chicago, Illinois
Dr. Robert J. Hay, American Type Culture Collection, Rockville, Maryland
Dr. O. Hayaishi, Keio University, Tokyo, Japan
Dr. C. H. Heathcock, University of California, Berkeley, California
Dr. Steven S. Hecht, Naylor Dana Institute, Valhalla, New York
Dr. Charles Heidelberger, University of Southern California Comprehensive Cancer Center, Los Angeles, California
Dr. Allen Heim, Food and Drug Administration, Rockville, Maryland

Dr. R. Marian Hicks, Middlesex Hospital Medical School, London, England
Dr. Gavin Hildick-Smith, Johnson & Johnson, New Brunswick, New Jersey
Dr. Donald L. Hill, Southern Research Institute, Birmingham, Alabama
Dr. Jane Hixson, Southern Research Institute, Brimingham, Alabama
Dr. Dietrich Hoffman, American Health Foundation, Valhalla, New York
Dr. Paul Howard-Flanders, Yale University, New Haven, Connecticut
Dr. Eliezer Huberman, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Dr. Yuzuru Ishimura, Keio University, Tokyo, Japan
Ms. Lois J. Jacob, University of Wisconsin, Madison, Wisconsin
Dr. Alan Jeffrey, Columbia University, New York, New York
Dr. Allen Jefferys, Institute of Cancer Research, New York, New York
Dr. Donald M. Jerina, NIAMDD, NIH, Bethesda, Maryland
Dr. Gottfried Kellermann, University of Wisconsin, Madison, Wisconsin
Dr. Paul Kleinhues, Max Planck Institute für Hirnforschung, Cologne, Germany
Dr. Hynda Kleinman, NIDR
Dr. Masahiko Kodama, National Cancer Center Research Institute, Tokyo, Japan
Dr. Margaret Kripke, Frederick Cancer Research Center, Frederick, Maryland
Dr. Toshio Kuroki, University of Tokyo, Tokyo, Japan
Dr. Irwin Leav, Tufts Medical School, Boston, Massachusetts
Dr. Stephen Lesko, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Wayne Levin, Hoffmann-La Roche, Nutley, New Jersey
Dr. Synnøve Liaaen-Jensen, University of Trondheim, Trondheim, Norway
Dr. Michael Lieberman, Research Triangle, Chapel Hill, North Carolina
Dr. Steward Linn, University of California at Berkeley, Berkeley California
Dr. John C. Linnell, Vincent Square Laboratories of Westminster Hospital, London, England
Dr. Martin Lipkin, Memorial Sloan-Kettering Cancer Center, New York, New York
Dr. Seymour R. Lipsky, Yale University, New Haven, Connecticut
Dr. Robert S. H. Liu, University of Hawaii, Honolulu, Hawaii
Dr. J. William Lloyd, United Steel Workers of America, Washington, D.C.
Dr. Lawrence A. Loeb, Institute for Cancer Research, Philadelphia, Pennsylvania
Professor Nicola Loprieno, Laboratorio Di Mutagenesi E Differenziamento, Pisa, Italy
Dr. Ronald Lorentzen, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Robert Lyle, University of New Hampshire, Durham, New Hampshire
Dr. Peter Magee, Temple University, Philadelphia, Pennsylvania
Dr. Heinrich Malling, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
Dr. James A. Marshall, Northwestern University, Evanston, Illinois
Dr. Hidetsuru Matsushita, National Institute for Industrial Health, Tokyo, Japan
Dr. H. Mayer, F. Hoffmann-La Roche and Company, A. G., Basel, Switzerland
Dr. Justin McCormick, Michigan State University, East Lansing, Michigan
Dr. Martin L. Meltz, Southwest Foundation for Research and Education, San Antonio, Texas
Dr. George Michalopoulos, University of Wisconsin, Madison, Wisconsin

Dr. Robert Miller, Clinical Epidemiology Branch, DCCP, NCI
Dr. George E. Milo, Ohio State University, Columbus, Ohio
Dr. Stephan Mironescu, American National Red Cross, Bethesda, Maryland
Dr. Ruggero Montesano, International Agency for Research on Cancer, Lyon, France
Dr. Richard C. Moon, ITT Research Institute, Chicago, Illinois
Dr. Tamotsu Morita, Shizuoka University, Shizuoka, Japan
Dr. Gerard P. Moss, Queen Mary College, London, England
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Dr. Chikayoshi Nagata, National Cancer Center Research Institute, Tokyo, Japan
Dr. Koji Nakanishi, Columbia University, New York, New York
Dr. Daniel W. Nebert, NICHHD, NIH, Bethesda, Maryland
Professor Giovanni Neri, Catholic University, Rome, Italy
Dr. Paul Nettesheim, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Dr. Warren Nichols, Institute for Medical Research, Camden, New Jersey
Dr. Taisei Nomura, Osaka University Medical School, Osaka, Japan
Dr. Stephen O'Brien, NCI
Dr. Shigeyoshi Odashima, National Institute of Hygienic Sciences, Tokyo, Japan
Dr. Thomas Oesterling, Johnson & Johnson, New Brunswick, New Jersey
Dr. Norbert P. Page, National Institute of Occupational Safety and Health, Rockville, Maryland
Dr. Beverly Paigen, Roswell Park Memorial Institute, Buffalo, New York
Dr. Robert B. Painter, University of California at San Francisco, San Francisco, California
Mr. Kenneth Palmer, Food and Drug Administration, Washington, D.C.
Dr. Ismail Parsa, State University of New York, Brooklyn, New York
Dr. J. Paust, BASF Aktiengesellschaft, Ludwigshafen, Germany
Dr. Beverly A. Pawson, Hoffman-La Roche Inc., Nutley, New Jersey
Dr. Andrew Peterson, Lac Usc Cancer Center, Los Angeles, California
Mr. John Pezzuto, New Jersey Medical School, Newark, New Jersey
Dr. Roman J. Pienta, Frederick Cancer Research Center, Frederick, Maryland
Dr. Hans Popper, Mt. Sinai School of Medicine, New York, New York
Dr. Carl Povlsen, Pathological Anatomical Institute, Copenhagen, Denmark
Dr. Michael Prival, Food and Drug Administration, Washington, D.C.
Dr. Bernard Pullman, Institut de Biologie Physico-Chemique, Paris, France
Dr. Marilyn A. Rasco, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas
Dr. Janardan K. Reddy, Northwestern University, Chicago, Illinois
Dr. James Regan, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Dr. John J. Roberts, Chester Beatty Research Institute, Chalfont-St. Giles, Buchs., United Kingdom
Dr. Herbert S. Rosenkranz, New York Medical College, Valhalla, New York
Dr. Steven Roth, Johns Hopkins University, Baltimore, Maryland
Dr. Leo Sachs, Weizmann Institute of Science, Rehovot, Israel
Dr. Gordon Sato, University of California-San Diego, La Jolla, California

Dr. Ryo Sato, Osaka University, Osaka, Japan
Dr. Heinrich K. Schnoes, University of Wisconsin, Madison, Wisconsin
Dr. J. Schribner, Fred Hutchinson Cancer Research Institute, Seattle, Washington
Dr. Albert Segaloff, Alton Ochsner Medical Foundation, New Orleans, Louisiana
Dr. Howard Seliger, Johns Hopkins University, Baltimore, Maryland
Dr. Irving J. Selikoff, Mount Sinai School of Medicine, New York, New York
Dr. Martin F. Semmelhack, Cornell University, Ithaca, New York
Dr. Richard Setlow, Brookhaven National Laboratory, Brookhaven, New York
Dr. Sydney Shain, Southwest Foundation for Research and Education, San Antonio, Texas
Dr. K. Barry Sharpless, Massachusetts Institute of Technology, Cambridge, Massachusetts
Dr. Charles R. Shaw, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas
Dr. Fulmer Y. Shealy, Southern Research Institute, Birmingham, Alabama
Dr. Thomas Shelley, Johnson & Johnson, New Brunswick, New Jersey
Dr. Thomas Slaga, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Dr. Robert A. Squire, The Johns Hopkins University, Baltimore, Maryland
Dr. Peter M. Steinert, NCI
Dr. Bernard Stewart, The University of New South Wales, Kensington, New South Wales, Australia
Dr. Bernard Strauss, University of Chicago, Chicago, Illinois
Dr. Henry Stroble, University of Texas Medical School, Houston, Texas
Dr. Takashi Sugimura, National Cancer Center Research Institute, Tokyo, Japan
Dr. Gonya Takahashi, Kyoto University, Kyoto, Japan
Dr. Wolfgang Thon, Deutsches Krebsforschungszentrum, Heidelberg, Germany
Dr. Lorenzo Tomatis, International Agency for Research on Cancer, Lyon, France
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Dr. Benjamin F. Trump, University of Maryland, Baltimore, Maryland
Dr. John C. M. Tsibris, University of Florida, Gainesville, Florida
Dr. Paul Ts'o, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Tokeki Tsutsui, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Dr. Willie Turner, Howard University, Washington, D.C.
Dr. Stephen Unger, Palo Alto, California
Dr. J. L. Van Lancker, Center for the Health Sciences, University of California at Los Angeles, Los Angeles, California
Dr. Elliot Vesell, Hershey Medical Center, Hershey, Pennsylvania
Dr. Wolfgang Wechsler, Max Planck Institut für Hirnforschung, Cologne, Germany
Dr. I. Bernard Weinstein, Columbia University, New York, New York
Dr. Steven C. Welch, University of Houston, Houston, Texas
Dr. Sefton R. Wellings, University of California, Davis, California
Dr. Gary Williams, American Health Foundation, New York, New York
Dr. Evelyn Witkin, Rutgers University, New Brunswick, New Jersey
Dr. Todd Wipke, University of California, Santa Cruz, California

Dr. David Wulfman, University
of Missouri at Rolla, Rolla,
Missouri
Dr. Errol Zeiger, National
Institute of Environmental
Health Sciences, Research
Triangle Park, North Carolina

3. SCIENTIFIC ACTIVITIES: PROGRESS HIGHLIGHTS

The Carcinogenesis Program covers a broad front of attack towards the identification of causative factors and the prevention of cancers. Work in chemical carcinogenesis is characterized to a large extent by the multi-year duration of lifespan studies in laboratory animals, with a resultant long-range commitment of efforts and resources.

A major new emphasis is being given to studies of carcinogen effects by in vitro methods focusing on biochemical interactions, mutagenic effects, and neoplastic transformation of cells in culture. Many other projects represent interrelated research approaches aimed at developing and defining research methods for study of the chemical, biochemical, biological, and molecular mechanisms responsible for the host response to chemical and physical carcinogens, and for the identification of exploitable preventive measures. Many of the individual projects in this coordinated program have been underway for several years and represent a patient, intensive, and costly long-term effort towards the attainment of specific program goals.

The Program's scientific accomplishments derive both from the intramural laboratories and from contract-supported projects; these cover fundamental research on the basic mechanisms of carcinogenesis, model and method developments, cancer pathogenesis studies, identification of carcinogens, and development of resources. Summary reports are provided by the Branches and segments making up the Operational Units.

Major accomplishments have been reached during this fiscal year. Highlights are summarized here in the perspective of the overall activity of the Program by research thrust rather than by organizational components.

A. BIOASSAYS FOR CARCINOGENIC ACTIVITY

A large-scale national resource has been developed to submit a large number of chemicals to long-term carcinogenicity bioassays in laboratory animals in well-defined and standardized experimental conditions. Approximately 335 chemicals are currently being screened for carcinogenicity. Approximately 125,000 animals were used this year for bioassay operations. These include about 55,000 rats of the Osborne-Mendel, Fischer/344, and Sprague-Dawley strains; 55,000 mice of the Swiss, C57Bl/6 and hybrid B6C3F1 strains; and 15,000 animals of other species, mostly hamsters. Most chemicals are tested by a protocol that includes preliminary toxicity studies followed by life-time feeding tests on groups of 50 animals each in two species, both sexes, and at two dose levels (including a dose close to the maximum tolerated dose).

Guidelines have been established for standardized, detailed protocols for the major bioassay procedures and a detailed pathology procedure has been established. These procedures were published and have already received wide acceptance by Government agencies, industry, and also in foreign countries. With these developments the Program has made an important transition. Until recently, bioassays were primarily aimed at the screening of environmental chemicals to identify suspected carcinogens for further study. The present

generation of detailed data is at a level of sophistication and control needed to assist agencies which are responsible for the making of regulatory decisions; these detailed bioassays also provide a solid scientific basis of evidence on the identification of chemicals as carcinogens.

The day-to-day management of an expanding program of this magnitude is an extremely demanding task. The demands placed on NCI personnel by this task were so extreme that a prime contract was established for bioassay management in March 1974. This contract with Tracor Jitco, Inc. (NO1-CP-43340, Bioassay Operations Segment), monitors all aspects of the bioassay program including experimental design, planning of resources (materials, animals, laboratory facilities), conduct of studies, data collection, and analysis and presentation of results. The prime contractor negotiated and assumed responsibility for the operation of the bioassay laboratories as subcontracts. Considerable attention was given to the development of inhalation bioassay resources. A draft of guidelines for such tests was prepared by staff and an evaluation of needs at the Department level was made on this issue. Meanwhile, a large new facility for inhalation studies was developed by New York University at the Sterling Forest Laboratory.

In the future, increased emphasis will be placed upon mechanisms involved in the carcinogenic effects in test animals, and in evaluating the relationship of level of carcinogen exposure to these mechanisms. This will involve more sophisticated analyses in sub-chronic dose finding studies, and, in some cases, the inclusion of additional dosage levels in chronic studies; where appropriate, metabolic and pharmacokinetic studies will be included. It is hoped that such information will assist in extrapolating possible carcinogenic risks from animal test results to man.

In Vitro Program

Specific project areas within this program are (1) development and validation of mammalian-cell transformation systems; (2) validation and utilization of microbial mutagenesis assays; (3) development and validation of mammalian cell mutagenesis systems; and (4) development of more effective and efficient cell culture systems through continuing research efforts in the Cancer Research Emphasis Grants (CREG) program.

Studies are now underway to standardize and determine the predictive value of transformation systems using the BALB/3T3 cell line, primary and early passage Fischer rat embryo cells, Fischer rat embryo cells infected with Rauscher leukemia virus, hamster embryo cells treated either directly in culture or exposed to the carcinogen transplacentally, and liver, skin and mammary gland epithelial cells. Each system is first being standardized with respect to a definition of the methodology and determination of reproducibility of the assay. Efforts are also being directed to assessing the value of endpoints other than morphological transformation, defining appropriate metabolic activation systems and increasing the sensitivity of the assays. Once the protocol for each system is defined, the predictive value of the assay is determined in double-blind studies, using known carcinogens, non-carcinogenic analogues, and non-carcinogens. Although there are reports in the literature on the use of all these systems, the development of

standardized protocols is a time-consuming process and all the systems mentioned, except the one using hamster embryo cells, are still in the development phase.

Studies with a clonal assay using hamster embryo cells have been underway for a considerably longer period of time and a total of 75 carcinogens and non-carcinogenic analogues have been tested. These include direct alkylating agents, polycyclic hydrocarbons, nitrosamines and amides, aromatic amines and aminoazo dyes and a variety of miscellaneous compounds. The data show excellent correlation between the ability of the chemicals to transform hamster embryo cells and their reported carcinogenic activity. When the total number of chemicals is considered, none of the non-carcinogenic analogues induced transformation and 46 of the 54 carcinogens were positive.

Proposals are now being evaluated for mutagenicity studies with microbial and mammalian cell systems and awards will be made shortly. A correlation between mutagenicity and carcinogenicity has been demonstrated, but it was considered that there was a need for a broad-based assessment of the predictive value of the systems through coordinated studies conducted in several laboratories. Selected chemical compounds will be tested double-blind in eight strains of microorganisms with and without metabolic activation. The microorganisms to be used are the five Salmonella strains developed by Dr. Ames and three E. coli strains. The metabolic activation systems will be derived from the livers of Fischer rats, the B6C3F1 mouse which is the hybrid progeny of the C57B1/6 (female) by the C3H/He (male) and Syrian hamsters. All these animals are currently used in the NCI bioassay program. Final, critical statistical evaluation of the data obtained in this study will provide guidelines for standardizing the test system. The mammalian mutagenesis system to be developed and validated uses the thymidine kinase locus in L5178Y cells. One of the important components of these contracts will be to provide support to the Chemical Selection Committee by prescreening compounds under consideration.

The first series of CREGs in in vitro chemical carcinogenesis have been selected. The objective is to study the interaction of chemical carcinogens and mammalian cells in vitro with emphasis on:

(1) Development of new model systems for neoplastic transformation including the use of human and non-human primate cells;

(2) Development and/or refinement of methodology for the early identification and quantitation of neoplastic transformation, including biochemical, cytological or immunological markers; and

(3) Development and characterization of new approaches to the metabolic activation of carcinogens and pro-carcinogens which can be applied to in vitro systems.

Carcinogenesis Bioassay Data System (CBDS) and Technical Reports

Emphasis continues to be placed on the collection of detailed information on all aspects of a carcinogenesis bioassay. A system, designed and implemented

by intramural staff to accomplish this collection as well as the related storage and retrieval function, receives data input on experimental designs, clinical observations, survival, and pathology results from about 20 laboratories. The route data entry into CBDS is provided under contract (Contractor: EG&G/Mason Research Institute, NO1-CP-43257, Bioassay Operations Segment). The system is being extended to facilitate data analysis using the best available techniques.

Data collected from bioassay studies are important in formulating public policy pertaining to the use of the agents tested. It is necessary that the results of these studies be properly recorded and analyzed so that they may be effectively used in the decision-making process.

One of the most pressing concerns in the bioassay program is a need to publish a full documentation of each bioassay with all the details of the methodology, conduct of the test, and results. While reports in open literature or conferences will still be encouraged, the NCI will report separately the result of each compound tested for carcinogenicity.

The first two reports were published in March 1976. *Technical Report No. 1* contains the "Guidelines for Carcinogenesis Bioassay in Small Rodents", as mentioned previously, while *No. 2* contains the first published fully detailed bioassay on trichloroethylene. Approximately 60 additional reports have been prepared; 11 reports listed in the section on the Bioassay Operational Unit have been published in the technical report series. Procedures for the publication of final bioassay reports in the format of journal articles and of reports of preliminary findings have been worked out and approved by the Assistant Secretary of Health. Efforts are currently underway to modify the existing data system to permit more rapid retrieval and monitoring on current data. Information management and on-line computerized systems are under consideration.

Chemicals Under Test

The main categories of compounds on test are pesticides and related chemicals, drugs and cosmetics, chemical intermediates and reagents, dyes and related chemicals, solvents, food or food additives, and rubber and paint chemicals.

The large expansion in the number of chemicals placed on test in FY 1972-1973 is now generating a large workload in pathology evaluation, data analysis, and reporting. Approximately one-half of the chemicals on test are now in these last three phases.

The testing activities, contributed from the laboratories coordinated by the prime contractor as well as from research institutes and university laboratories, provide much needed new information on the carcinogenic potential of chemicals to which the population is exposed. They also greatly contribute to the development and evaluation of research methods and of pathology criteria relevant to the whole field of studies on carcinogenesis and cancer prevention. A list of chemicals on test was prepared for Congress on June 14, 1977. This list appears in the appendix to this report.

B. CHEMISTRY, METABOLISM, AND MOLECULAR INTERACTIONS OF CARCINOGENS

The understanding and the possible inhibition of chemical carcinogenesis are based on characterizing the critical interactions that enable certain chemical agents to induce a neoplastic cell change. Elucidation of these chemical mechanisms has a profound influence at several levels: on the understanding of the nature of the cancer induction process; on the identification of selective inhibitory mechanisms capable of blocking carcinogenic events, on the identification of susceptible states, on the development of specific markers of carcinogenesis, on the characterization of metabolic pathways of carcinogen activation that can be used to define species susceptibility, on the identification of chemical structure-activity relationships which can become predictive indicators of carcinogenic activity, and finally on the design of specific bioassay conditions capable of revealing the potential effect of carcinogens.

Mechanism studies have already been the key for the development of current short-term bioassay prescreen methods: knowledge of the metabolic requirements for carcinogen activation has led to the use of the appropriate enzyme systems for mutagenesis and transformation tests. Knowledge of carcinogenesis mechanisms has already made it possible to predict the likelihood of hazards for certain classes of environmental chemicals, and also to develop current assays on the susceptibility of carcinogens in humans. The work on mechanisms should therefore be viewed not as an isolated research endeavor but as an integral part of the attack on the problem of cancer induction and prevention with ultimate impact on the problem of human cancer.

Polycyclic Aromatic Hydrocarbons

The discovery last year of the exact stereochemical structure of a metabolite of benzo[a]pyrene (BP) responsible for the majority of the in vivo BP-DNA reaction and possessing an extremely high mutagenic activity (Contractors: Institute for Cancer Research [Chester Beatty Research Institute], NO1-CP-33367, and Weizmann Institute of Science, NO1-CP-02217, Chemistry and Molecular Carcinogenesis Segment) was a major advance, particularly for structure-activity studies involving polycyclic aromatic hydrocarbons. Using enzyme preparations from mammalian cells, the formation of r-7, t-8, dihydroxy-t-9, 10-oxy-7,8,9,10-tetrahydro-BP or the anti form of the 7,8 diol-9,10 epoxide of benzo[a]pyrene was shown. The detailed mechanism of the enzymatic formation of this highly mutagenic metabolite has now been elucidated by the intramural laboratories of the Chemistry Branch. This diol-epoxide is also the most potent carcinogen among BP derivatives in experimental animals, further supporting the suggestion that this may be the ultimate carcinogenic form of benzo[a] pyrene. Further work (Contractor: Weizmann Institute of Science, NO1-CP-02217, Chemistry and Molecular Carcinogenesis Segment) has shown that cell transformation by BP is due to a mutation and that this mutation can occur in one out of a small number of genes. At the molecular level, most of the diol-epoxide metabolite is found bound to guanosine residues, but a small

fraction is bound to adenine (see Chemistry Branch) causing a local denaturation of the DNA chain susceptible to the action of a single-stranded nuclease. This type of binding, hitherto unreported, may suggest that the epoxide may attack the phosphorous diester bond directly, in addition to modifying the DNA by binding to the bases.

A series of metabolites of 5 polycyclic aromatic hydrocarbons, including the isomeric forms of the diol-epoxide of B[a]P, have been synthesized (Contractors: Midwest Research Institute, NO1-CP-33387, and University of Chicago, NO1-CP-33385, Chemistry and Molecular Carcinogenesis Segment) and made available to the research community through the Standard Carcinogen Reference Repository (Contractor: IIT Research Institute, NO1-CP-55646, Information and Resources Segment). Many are now available with radiolabeling (^{3}H and ^{14}C).

N-Nitroso Compounds

The extensive research program developed in recent years on the formation, metabolism, analytical identification, and biological activity of this important group of carcinogens continued to make remarkable progress.

A major program on the carcinogenic activity, formation, metabolism, and analytical methods for nitrosamines and related compounds is now well underway. Particular emphasis is being given to the possible in vivo formation of nitrosamines, the metabolic fate of the nitrosamines, their reaction with target cell constituents, and their possible involvement in tumor development in humans (see Metabolism and Toxicology Branch and Chemistry Operational Unit).

Analytical methods for elucidating the environmental distribution of N-nitroso compounds and for understanding the biochemical and biological consequences of exposure to them are rapidly improving. A coordinated program is continuing in close collaboration with staff in FDA who have a direct concern with analytical resources in this area. This program includes activities concerned with (a) improved methodologies for the analysis of volatile and non-volatile nitrosamines, (b) development of group-specific analytical methodologies suitable for the analysis of total nitrosamine content, (c) improvement of instrumentation in this analytical area, and (d) preparation and distribution of labeled nitrosamines for the validation of previously developed analytical methods (see report from the Carcinogen Metabolism and Toxicology Segment of the Chemistry Operational Unit). Studies in this area are being pursued in-house and through other collaborative projects (Contractors: University of Nebraska [Eppley Institute], NO1-CP-33278, and ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director).

Particular attention was given to the analysis of volatile nitrosamines and to methods for monitoring them in the air. The possible formation of nitrosamines in the environment, and particularly to foodstuffs during preparation and storage or from endogenous materials consumed with the diet is being further investigated (Contractor: Massachusetts Institute of Technology, NO1-CP-33315, Carcinogen Metabolism and Toxicology Segment). Gastric nitrite was shown to

be elevated to hundreds of parts per million in some individuals, and it appears that high stomach pH is essential for high nitrite. Biological effects and metabolic characteristics of many N-nitroso compounds are being elucidated in studies on tissue and cellular pathology throughout the Program. Nitrosamines are usually considered to be systemic carcinogens and to act in tissues remote from the application site. Recent bioassay results however have demonstrated that nitrosamines with specific molecular structures are primarily locally acting carcinogens (Contractor: University of Nebraska [Eppley Institute], NO1-CP-33278, Office of the Associate Director).

Other Carcinogens

Several additional classes and types of carcinogens are studied throughout the Program from the chemical, biological, and metabolic point of view, including aflatoxin and other mycotoxins, aromatic amines, anthraquinones, chlorinated hydrocarbons, substituted hydrazines, metal derivatives and others. These and other carcinogens are provided in carefully monitored purity as reference standards through the Standard Carcinogen Reference Repository (Contractor: IIT Research Institute, NO1-CP-55646, Interdisciplinary Programs and Resources Operational Unit).

Molecular Control Mechanisms

Studies on the binding of carcinogens to target macromolecules and studies on the effects of carcinogens on DNA repair synthesis have continued both in the intramural laboratories (Chemistry Branch, Experimental Pathology Branch) and in the collaborative program (Chemistry Operational Unit and Biology Operational Unit; Contractor: ERDA-Oak Ridge National Laboratory, YO1-CP-50200, Office of the Associate Director).

New observations on hydrogen ion transport through the energy-transforming systems of the respiratory chain in animal cell mitochondria force a drastic revision of the long-standing chemi-osmotic theory of energy recovery from oxidation of cell nutrients (Contractor: The Johns Hopkins University, NO1-CP-45610, Chemistry Operational Unit). These observations on the hydrogen ion pump may radically change the course of research on bioenergetics of normal and tumor mitochondria.

Additional progress was reported in studies on the following areas: surface membrane transport systems, specific synthetic pathways regulating cell functions and their inhibition, biochemical role of vitamin A on gycoprotein synthesis, and the role of nutritional deficiencies (e.g., lipotropes) in carcinogenesis.

An area which has rapidly developed is that of anticarcinogenesis: specific pharmacologic inhibitors of cell differentiation and carcinogenesis have been developed and studied in various cellular and animal model systems. A group of synthetic compounds called retinoids has revealed a particularly high level of activity against the effect of carcinogens in several epithelial tissues (respiratory tract, bladder, mammary gland), showing pharmacologic activity

even at very low concentrations. The potential use of these new drugs for the selective inhibition of carcinogenesis is being actively investigated (Lung Cancer Branch and Lung Cancer Segment).

C. IN VITRO MODELS

FOR CHEMICAL CARCINOGENESIS

Chemical carcinogenesis research has gone through a methodological revolution in the last few years. This was due primarily to the development of methods (a) for the neoplastic transformation of cells in culture by chemical carcinogens, (b) for the detection of the mutagenic activity of carcinogens, and (c) for the identification of chemical and biological interactions of carcinogens with target macromolecules such as covalent binding to nucleic acids and DNA repair.

The rapid development and definition of these new methods in the last decade, and particularly in the last four or five years, has been extensively stimulated and supported by the Carcinogenesis Program. In fact, a large proportion of the whole field of carcinogenesis research is now focusing on in vitro methods. Direct interaction of carcinogens with target macromolecules in vitro, in mutant organisms or in mammalian cells in culture can now be studied and related to the induction of tumors in vivo by virtue of the fact that pathways of metabolic activation of carcinogens have been discovered and defined. These advances have led to the development of methods for carcinogen activation which have revealed the biological activity in vitro of agents whose in vivo effects were known but could not be reproduced in vitro without metabolic activation. Another major factor in the progress of in vitro approaches to carcinogenesis studies has been the development of cell culture methods coupled with methods for cellular genetic studies.

The development of this component of carcinogenesis research can hardly be described separately from the remainder of the field, since the methods for in vitro study are by now thoroughly integrated into all aspects of the program, such as cellular interactions, species specificity, tissue specificity, metabolic pathways, role of nucleic acids, role of membranes, relationships between mutagenic and carcinogenic events, and study of early lesions in carcinogenesis of human tissue responses of carcinogens.

The new in vitro methods have a major characteristic in common: they are short-term methods, so that the effect of carcinogens on target macromolecules on mutagenesis and on neoplastic transformation of cells in culture can be measured in a time-frame going from a few days to a few weeks (at times extending to a few months for the demonstration of neoplastic growth after inoculation of transformed cells in animals). In comparison with the time for in vivo animal studies, usually three years or more, this short time-frame represents a major breakthrough.

The key question, obviously, is whether the results of these short-term methods can be considered equivalent or predictive of the actual induction

of tumors in the whole animal or humans. The question is crucial both for the future use of short-term methods for carcinogen testing and for their use as a basis for studies on mechanisms, on host susceptibility and on the inhibition of carcinogenesis.

The in vitro methods of cell transformation can be extended to human cells and this represents the only usable approach to a direct experimentation of the activity of carcinogens on human tissues. The gap between animal and human data in evaluating susceptibility to carcinogens can thus be bridged. New preliminary results show that in fact human cells can be transformed in culture by carcinogens.

Transformation of a cloned line of human fibroblasts was observed following treatment with 4-nitroquinoline-1 oxide and N-methyl-N'-nitro-N-nitroso-quanidine. Transformants were identified by morphology, growth in soft agar, and production of tumors in nude mice. The frequency of transformants was very low and required growth in culture for 6-8 weeks for detection (Chemistry Branch).

In addition, organ culture of several human tissues has been developed in the last two years to include studies on bronchial, intestinal and pancreatic epithelia; the binding of carcinogens, their metabolic requirement and localization have been demonstrated directly in human target tissues treated in culture. The observed wide interindividual spread of binding levels (70-fold for the binding of benzo(a)pyrene to DNA in human bronchi) underscores the need to define both chemical exposure and host susceptibility factors (Experimental Pathology Branch).

Because of the great importance of these new methods in the identification and evaluation of carcinogens, it has been widely recognized by the scientific leaders in the field that an essential condition of progress in this area is that these methods be well defined and validated, particularly in terms of reproducibility and of comparability with in vivo effects.

In planning a concerted effort in this direction, over the last few years, several factors were recognized. (a) Only a few systems were established with a sufficient degree of methodological definition (e.g., for mutagenesis, the test in Salmonella mutant strains developed by Ames; for transformation, the hamster embryo cell system developed by Sachs, DiPaolo and co-workers. (b) Several other systems were in an advanced stage of development (e.g., transformation of mouse C3H 10T½ cells developed by Heidelberger and several epithelial cell systems). (c) Methods for metabolic activation were essential in conjunction with the test systems (using microsomal preparations, co-cultivation of metabolically active cells or host-mediation). (d) The response of the in vitro systems had to be validated against a large number of chemicals, well defined for their carcinogenic activity in whole animals (including procarcinogens, active metabolites, and noncarcinogens) to determine the extent of analogy between in vitro and in vivo effects. (e) Double blind tests were needed to establish the degrees of reproducibility and objectivity of the tests. (f) Criteria were needed for the recognition of neoplastic transformation of cells in culture, with particular attention

to those applicable to epithelial cells and to human cells. (g) A battery of cell systems should be evaluated, and epithelial cell systems should be included, particularly in view of the fact that most tumors arising in vivo in animals and humans are tumors derived from epithelial tissues. (h) The interrelation of mutagenic and carcinogenic events had to be further defined and possibly studied within the same cells. (i) The possible interactions of viral, physical, and chemical agents in cell transformation needed elucidation.

These and other considerations led to the development of a broad-based effort throughout the Carcinogenesis Program to which all Branches and Segments have contributed. Extensive consultation and advice were received from leading investigators in the field individually and in the course of several workshops organized for this purpose.

Over 40 contracts support projects on in vitro carcinogenesis methods and studies throughout the Carcinogenesis Program. Extensive work is conducted throughout the intramural laboratories. Because of this total involvement of the Program, activities strictly identified with this field are reported from most Branches and Segments. No separate segment was established for this work because much of it was being developed as an integral part of other existing Segments. However, an extensive group of additional projects has been developed for funding directly from the Office of the Associate Director. In addition, a first group of Cancer Research Emphasis Grants (CREGs) was requested by this Office, reviewed by NIH's Division of Research Grants' study sections and by the National Cancer Advisory Board, and approved for funding by this Program.

At least three main interrelated components can be identified in this program area: (a) the development, selection, definition and validation of a battery of methods for short-term carcinogen bioassay prescreening; (b) the development of new systems and the definition of the optimal conditions under which carcinogens are most effective in these systems, with emphasis on metabolic activation requirements, epithelial cells, human cells and markers of cell transformation; and (c) research on the mechanism of carcinogenesis, on susceptibility factors and on the inhibition of carcinogenesis. Each of these approaches has been pioneered in the Carcinogenesis Program over several years.

DNA Binding and Repair, Mutagenesis

Methods for the evaluation of DNA binding and repair were developed since the 1960s (intramural research; Contractor: ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory], YO1-CP-50200, Office of the Associate Director). Also, since the 1960s direct and host-mediated mutagenesis methods as applied to carcinogens were developed under Program support at a time when skepticism often prevailed on the relevance of mutagenesis methods to carcinogenesis research (Contractor: ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory], YO1-CP-50200; staff on this project later formed the nucleus of the National Institute of Environmental Health Sciences' mutagenesis program, with which close collaboration continues).

Following a workshop organized by the Bioassay Segment on Mutagenicity as a Prescreen for Chemical Carcinogens in July 1972, projects were developed for the first systematic effort at validation of short-term prescreening tests. It was found that, while mammalian cell systems need further development and validation, the bacterial mutagenesis systems showed a high degree of correlation with carcinogenicity. These studies have provided a substantial contribution to the validation of the Ames' test and to the further definition of its sensitivity; this test has now been extensively used in academic and industrial laboratories. Further validation of mutagenesis tests is planned. Much current progress is being made in the study of DNA repair following carcinogen exposure. It was found that treatment of cells or viruses with benzo(a)pyrene diol-epoxides and other carcinogens caused extensive damage to the DNA which was repaired by an excision-repair mechanism. In view of the high mutagenic activity, it is conceivable that benzo(a)pyrene 7,8-diol-9,10 epoxides may be ultimate, metabolically activated forms of benzo(a)pyrene (see Chemistry Branch, Experimental Pathology Branch, Chemistry and Molecular Carcinogenesis Segment, Biological Models Segment, and Office of the Associate Director).

Neoplastic Transformation of Cells in Culture

Much new work was developed in this area. The hamster embryo cell transformation system as developed by DiPaolo was used to establish a modified technique so that transformed foci can be observed on a normal cell background, thus eliminating the need to examine every colony in detail for transformation (Biology Branch).

A systematic validation of the host-mediated assay showed an almost complete correlation between in vitro transformation and known in vivo activity in a series of 88 compounds tested under a code; (Contractor: NCI-FCRC, NO1-CO-25423, Office of the Associate Director). The transplacental assay system developed by DiPaolo for identifying the oncogenic potential of chemicals is currently being used with modified criteria for assessing transformation (Contractor: ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory], YO1-CP-50200). It was found that a human osteosarcoma line which did not induce tumors in nude mice was transformed by chemical carcinogens to a more neoplastic form which induced tumors in mice (Carcinogenesis Metabolism and Toxicology Branch).

The transformation of syrian hamster cells by chemical carcinogens was found to be direct and not due to activation of an endogenous virus. Analysis of carcinogen-treated guinea pig cells during the stages of carcinogenesis revealed that lymphotoxin susceptibility develops in close proximity to the time of neoplastic transformation (Biology Branch).

It has been found that whereas plasminogen activator (PA) production is a useful marker for the in vitro transformation of fibroblast cultures it is not a reliable predictor of the tumorigenic potential of rat/liver epithelial cell cultures. The tumor-promoting agent 12-O-tetradecanoyl phorbol-13-acetate, a potent inducer of PA production in cell culture, may provide a rapid bioassay and an in vitro system for the elucidation of the mechanism(s) of promoting agents (Contractor: Columbia University, NO1-CP-23234, Interdisciplinary Programs and Resources Operational Unit).

In the past year, a technique has been perfected which allows the transfer of one or a few chromosomes from donor to recipient cells. This technique may be useful in identifying and characterizing genetic factors involved in the malignant transformation of cells by chemical carcinogens (Contractor: Yale University, NO1-CP-55673; Chemistry Operational Unit).

A battery of test systems is currently under validation in a series of parellel projects, using the following cell types: Fischer 344 rat embryo cells (Contractor: A.D. Little, Inc., NO1-CP-55711, Office of the Associate Director); the same with Rauscher leukemia virus (Pfizer, Inc./ J. L. Smith Memorial, NO1-CP-55703, and Microbiological Associates, Inc., NO1-CP-55670); Balb/c 3T3 cells (Contractor: A.D. Little, Inc., NO1-CP-55711); hamster embryo cells by host-mediated treatment (Contractor: Stanford Research Institute, NO1-CP-55701).

Other cell systems are being developed in other projects: liver and mammary epithelial cells (Contractor: American Health Foundation, NO1-CP-55705); diploid skin epithelial cells (Contractor: Children's Hospital of Akron, NO1-CP-55706); liver epithelial cells (Contractor: University of North Carolina, NO1-CP-55707). Work on epithelial cell systems continues at Columbia University (NO1-CP-23234) and at the International Agency for Research on Cancer (NO1-CP-55630).

D. EXPERIMENTAL PATHOLOGY STUDIES OF CANCER INDUCTION AND PREVENTION

A major effort in the Carcinogenesis Program is devoted to studies designed to develop and define experimental models of the principal types of cancer and to define their pathogenesis at the tissue and cellular level.

These studies focus the study of carcinogenesis on the specific characteristics of the target tissues, in animals and in their human counterpart, as they relate to major types of human cancers. Both susceptibility and resistance to cancer induction are studied. A particular emphasis is given to a correlation of animal models with human cancers.

Lung Carcinogenesis

Respiratory tract carcinogenesis studies have continued to make significant progress. They include studies on high risk human groups, experimental animal bioassay and pathogenesis studies, organ culture studies both in animal and on human tissues, and morphological-biochemical correlation studies on mechanisms of carcinogenesis and their selective inhibition by anticarcinogens.

Respiratory carcinogenesis bioassay experiments have continued on several chemicals of environmental significance, by various techniques including inhalation and intratracheal instillation. Nitrogen dioxide inhalation may enhance the carcinogenic response to inhaled benzo[a]pyrene; a co-carcinogenic effect has been reported also for croton oil inhalation. Extensive new inhalation facilities were developed. In animal inhalation studies two

widely used industrial intermediates, dimethylcarbamoyl chloride and epichlorohydrin, have been found to cause squamous cell carcinomas of the nasal epithelium. These results suggest new industrial hazards of extreme importance. Concerned Federal agencies, the Director of the NCI Clearinghouse on Environmental Carcinogens, industries and unions have been notified. (Contractor: New York University, NO1-CP-33260, Biology Operational Unit).

Synergistic effects have been shown in hamsters in the induction of lung cancer between alpha radiation from Polonium-210 and the chemical carcinogen benzo[a]pyrene. Alpha radiation doses as low as 15-75 rads were found to produce lung cancer in hamsters (Contractor: Harvard University School of Public Health, NO1-CP-33273, Biology Operational Unit).

A new method was recently developed to induce tumors in a small predetermined segment of the respiratory tract in hamsters by delivering carcinogens through a special cannula. An improved method is being used in inhibition studies. (Lung Cancer Branch; Contractors: IIT Research Institute, NO1-CP-23292, Biology Operational Unit, and ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory], YO1-CP-50200, Office of the Associate Director). Inhibition by two retinoids in this model has already been shown for N-nitrosomethylurea-induced tracheal cancers (Contractor: ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory] see above).

The tracheal transplant method for carcinogen treatment of isolated tracheas grafted subcutaneously in isogenic animals was modified by treatment with carcinogen adsorbed on Lycra fibers. By this method, as little as 8 µg of 3-methylcholanthrene effectively induced tracheal carcinomas. If prior to transplanting, hamster tracheas are exposed in culture to crocidolite asbestos and 3-methylcholanthrene, less than 0.1 µg of carcinogen per four square millimeters of epithelial surface are required for anaplastic carcinoma induction. In replicate experiments, retinyl methyl ether at low concentrations inhibits squamous metaplasia and DNA synthesis in tracheal organ cultures after exposure to crocidolite and amosite asbestos. At these concentrations of the retinoid, cytotoxic changes were not observed in the epithelium (Contractor: University of Vermont, NO1-CP-33360, Biology Operational Unit).

Transformation of rat tracheal epithelium has been shown in an organ culture-cell culture system. Fully differentiated tracheal mucosa is first exposed in organ culture to MNNG followed by establishment of cell cultures to facilitate expression of neoplastic transformation. Studies of growth in soft agar and tumorigenesis show that neoplastic transformation occurs in cells derived from these carcinogen-exposed organ cultures. Other studies have shown that the earliest notable and persistent change of respiratory epithelium following brief in vivo exposure to carcinogen is the acquisition of an unlimited growth capacity in vitro. This occurs at various carcinogen dose levels following carcinogen exposure periods of 2-4 weeks and occurs 12-24 months before development of neoplasia. This alteration is thought to be one essential step in the development of neoplastic transformation (ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory], YO1-CP-50200, Office of the Associate Director).

Studies on inhibition of benzo[a]pyrene-induced pulmonary neoplasia in A/HeJ mice by the antioxidant butylated hydroxyanisole (BHA) continue to progress. Administration of BHA as little as four hours prior to B(a)P inhibits pulmonary tumors; and use of microsomes in vitro from these BHA-fed animals shows significantly reduced binding of BP metabolites to DNA. Analysis of high pressure liquid chromatography patterns indicates a decrease in formation of reactive metabolites and an increase in 3OH-B(a)P, the principal metabolite of detoxification. These results suggest that BHA may exert a direct effect on microsomal metabolism of B(a)P which results in a decreased carcinogenic potential of this polycyclic aromatic hydrocarbon (Contractor: University of Minnesota, NO1-CP-33364, Biology Operational Unit).

Organ and cell culture systems have been developed for the last several years in this program area and they are now extensively used to define the interaction of carcinogens and the stages of tissue response in the respiratory epithelium both in animals and in human tissues (Lung Cancer Branch and Experimental Pathology Branch; Contractors: ERDA-NCI Interagency Agreement [Oak Ridge National Laboratory], YO1-CP-50200, Litton Bionetics, Inc., NO1-CP-43274, University of Maryland, NO1-CP-43237, State University of New York [at Stony Brook], NO1-CP-33361, and University of Vermont, NO1-CP-33360, Lung Cancer Segment).

A major study of in vivo cell kinetics in normal, untreated adult hamster tracheal epithelium has been completed. Ciliated cells were not labeled nor observed in mitosis. Definitive values have been established for basal and mucus cells for lengths of S, G2, M, G1, cycle time, growth fraction, labeling index, and mitotic index (Contractor: Veterans Administration Hospital [Tampa], YO1-CP-55625, Biology Operational Unit).

The inhibition by retinoids of epithelial carcinogenesis continues to show great promise in several organ systems in long-term carcinogenesis experiments in defined animal models. It has been shown that retinyl acetate and retinyl methyl ether significantly reduce DMBA-induced benign and malignant breast tumors in the rat, and that retinyl acetate markedly inhibits the development of mammary adenocarcinomas induced by N-nitrosomethylurea. Further, studies show significant inhibition of bladder carcinogenesis induced by butyl-(hydroxybutyl)-nitrosamine in the Fischer rat (Contractor: IIT Research Institute, NO1-CP-23292, Biology Operational Unit; Lung Cancer Branch).

A number of new synthetic retinoids have shown a high degree of biologic activity in several in vitro test systems in reversing the effect of carcinogens. The binding requirements of these structures to a cellular retinoic acid binding protein have been investigated, and some pharmacologic properties ascertained. Recently, it was also shown that β-retinoic acid inhibits and reverses testosterone-induced lesions in mouse prostate organ cultures.

Colon Carcinogenesis

A program in this area was established in FY 1972 in close collaboration with the Field Studies and Statistics Program, DCCP, NCI, and with the National Large Bowel Cancer Project. Its focus is on the correlation of

colon cancer morbidity in migrant populations, the pathology of these cancers and environmental dietary factors. The role of bacterial flora in the metabolism of intestinal carcinogens and in the susceptibility of population groups is being studied. Because of the loss of staff experts and current personnel restrictions, much of this work is now being phased out, though certain new areas of experimental colon carcinogenesis are under development.

One hypothesis on the cause of human large bowel cancer suggests that intestinal bacteria metabolize chemical precursors to produce the actual carcinogens. Studies have continued on the characteristics and metabolism of bacteria isolated from the large bowel of individuals belonging to groups having differing risk for the development of colon cancer.

Analyses of the microbiological flora and neutral sterol composition of feces from individuals classified according to their statistical risk of developing colon cancer are being performed (Contractors: Virginia Polytechnic Institute and State University, NO1-CP-33334 and NO1-CP-55685, University of California at Los Angeles, NO1-CP-33380, and University of Missouri, NO1-CP-33335, Colon Cancer Segment). These investigations attempt to determine if certain microbiological or chemical markers for risk exist as well as if a specific microbial species may play a unique role in the carcinogenic process. A number of observations of interest have been made, but firm conclusions or hypotheses are still premature. A change in diet itself does not necessarily alter the microbial genera and species found in the feces. A low risk group appears to differ in relative quantities of different types of aerobic and anaerobic microorganisms from a high risk group. However, all types seem to be present in some degree irrespective of risk classification. High meat consumption increases the proportion of anaerobes. This is not mediated through high cholesterol intake since evidence indicates no increase in cholesterol or its metabolites was found in the excreted solids.

The association of Clostridium paraputrificum with risk group classification appears to be strengthened by measurement of quantitative levels of this bacterium in vegetarians and meat consumption populations. Studies on isolation and evaluation of the activity of this microbe appear warranted. Microorganisms have been isolated which require cholesterol for growth. Two major degradation products, coprostanol and ethyl-coprostanol, seem to be more highly esterified to long-chain fatty acids in a low risk population.

Colon cancer induction with azoxymethane (AM) or with 1,2-dimethyl hydrazine (DMH) has been potentiated in rats fed diets high in various types of fat (Contractor: American Health Foundation, NO1-CP-33208, Colon Cancer Segment). Such rats excreted greater quantities of cholesterol metabolites and bile acids. One such acid, deoxycholic acid, was a tumor promoter even in germ-free rats. DMH is less carcinogenic and AM is more carcinogenic in germ-free rats than in conventionally housed rats.

Results suggestive of a hereditary factor in skin fibroblasts in familial polyposis, phenotypically characterized by in vitro growth morphology, a possible marker for latent inherited polyposis, have been developed

(Contractor: Memorial Hospital for Cancer and Allied Diseases, NO1-CP-43366, Colon Cancer Segment). Fibroblasts from polyposis subjects were 100-1000 fold more susceptible to transformation (focus formation) with Kirsten murine sarcoma virus than normal skin cells.

In addition, efforts are devoted to establishing methods for the organ culture of human colon mucosa for the direct study of the interaction of carcinogens with this target human tissue (Experimental Pathology Branch; Contractor: Univ. of MD, NO1-CP-43237, Biology Operational Unit).

Pancreas Carcinogenesis

Exceptional progress has been made recently in the development of experimental models for the pathogenesis of pancreatic carcinoma and its study. The previously reported nitrosamine-induced hamster model has had particular success (Contractor: University of Nebraska [Eppley Institute], NO1-CP-33278, Office of the Associate Director). In this model, N-nitrosobis(2-oxopropyl)-amine (BOP) is a potent carcinogen. BOP induced, in a relatively short period of time, a high incidence of pancreatic ductal cell tumors in doses considerably lower than those needed with the previously demonstrated pancreatic carcinogen N-nitrosobis(2-hydroxypropyl)amine (BHP) and N-nitroso-bis(2-acetoxypropyl)amine (BAP). The pancreatotrophic effect of these three compounds was found to be directly related to the in vivo formation of N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP), which was formed more readily from BOP than from BHP and BAP. The assumption that HPOP may be a proximate pancreatic carcinogen was supported by the observation that N-nitroso-2,6-dimethylmorpholine, which has also been reported to be a pancreatic carcinogen, produced HPOP as an in vivo metabolite. The chemical structure of this metabolite is of specific interest, since it can occur in a cyclic form resembling the pyranose form of glucose (Eppley Institute, see above). The histopathogenesis of adenocarcinoma of the pancreas has also been investigated using this model. Results suggest that this cancer arises from pancreatic duct epithelium, progressing from duct hyperplasia to cystadenomas, to intraductal and invasive ductal carcinomas (Experimental Pathology Branch).

Studies of human pancreas cancers indicate that about 90 percent arise in duct epithelium, and may be classified into ten distinctive morphologic types. Marked atypia and carcinoma in situ of pancreas ducts were found in about 20 percent of patients with pancreas adenocarcinomas, suggesting that a significant latent period may exist between the onset of the recognizable neoplastic process and the appearance of invasion and symptoms (Contractor: Memorial Hospital for Cancer and Allied Diseases, NO1-CP-43232, Biology Operational Unit).

Human pathology studies have been developed to define the relationship of the pancreatic duct to the bile duct in the investigation of the bile reflux. Analysis to date indicates a trend toward an association of non-common channel ductal arrangements with abnormal pancreatic duct histology. A canine model for investigation of bile reflux indicates that reflux of a small amount of duodenal contents can occur shortly after feeding when mean

duodenal pressure can exceed mean pancreatic pressure (Contractor: Mayo Foundation, NO1-CP-55660, Biology Operational Unit). This new result is particularly interesting since biliary pancreatic reflux has also recently been reported in the monkey under relatively physiologic conditions (Contractor: University of Utah, NO1-CP-55709, Biology Operational Unit).

Human pancreatic duct has been maintained in organ culture for up to 60 days in explant culture with preservation of viability as measured morphologically and cytochemically (Contractor: University of Maryland, NO1-CP-43237, Biology Operational Unit). Such maintenance in a controlled experimental setting provides an excellent system to study the metabolism of chemical carcinogens. Preliminary results already indicate metabolism of polycyclic aromatic hydrocarbons by cultured human pancreatic duct. (Experimental Pathology Branch).

Much additional work has been accomplished towards the development of models and pathogenesis studies for carcinogenesis in the prostate, mammary gland, bladder, esophagus, and skin. The study of transplacental carcinogenesis has further defined models for the induction of kidney and nervous system tumors. The patas monkey is used to study transplacental mechanisms of carcinogen distribution and activity in primates with interesting results (Experimental Pathology Branch; Lung Cancer Branch; Contractor: Meloy Laboratories, Inc. NO1-CP-55613, BOU).

Of particular note has been extensive progress in organ culture maintenance and studies of metabolism of chemical carcinogens in culture of human bronchus. Many compounds, some known carcinogens, present in the environment and in tabocco smoke have been investigated (Experimental Pathology Branch).

E. SAFETY, TECHNICAL INFORMATION, AND PROGRAM RESOURCES

The Carcinogenesis Program requires supporting resources in several major areas. For example, the provisions of the National Cancer Act emphasize the need for a coordinated effort to collect, analyze, and disseminate data useful in the prevention, diagnosis, and treatment of cancer.

The rapidly growing interest of our society in the problems of cancer causation and prevention has required that a much more intensive effort be developed in the field of carcinogenesis documentation and resources. In this effort, close cooperation has been maintained with the other Federal agencies. Although the NCI has no responsibility for regulatory actions to accomplish the removal or reduction of exposure to carcinogens, the Carcinogenesis Program has continued to serve in an advisory role in the development of documentation for use by regulatory agencies in the Federal Government in relation to environmental carcinogens.

During this fiscal year, staff has participated in the DHEW Committee to coordinate Toxicology and Related Programs, in the interagency Collaborative Group on Environmental Carcinogenesis; in numerous meetings with staff from other agencies, such as DHEW, Food and Drug Administration, National Center for Toxicological Research, National Institute of Occupational Safety and Health, National Institute of Environmental Health Sciences, National Library of Medicine; Environmental Protection Agency; Department of Labor's Occupational Safety and Health Administration; Council on Environmental Quality, Energy Research and Development Administration, and others. Special emphasis was given to providing documentation and reviews requested by other agencies. International cooperation and liaison was developed further through participation in the activities of the International Agency for Research on Cancer and the US-Japan collaborative programs.

Chemical Repository

The Standard Chemical Carcinogen Reference Repository was established in July, 1975 to provide a centralized source for the safe storage and distribution of well-characterized potential carcinogens for use in cancer research all over the world. Besides commercial suppliers, repository stocks are received from various NCI synthesis programs and from surplus stocks of the Bioassay Program. Currently 175 compounds are stocked in the repository. Since the establishment of the repository, requests by over 2000 investigators have been met. For the past few months, approximately 300 requests for chemicals have been received monthly. Analytical and characterization data, as well as information on safe handling of each chemical, is provided with every shipment.

Conferences and workshops organized and/or sponsored by the Carcinogenesis Program

- Conference on Advances in Cancer Biology I—In Vitro Carcinogenesis, organized by Dr. U. Saffiotti and Dr. D. King, Jr. (Columbia University), was held at the Given Institute of Pathobiology, Aspen, Colorado, on July 18-22, 1976; sponsored by the University of Colorado.

- Retinoid Planning Workshop, organized by Dr. M. Sporn and Dr. A. Heim, was held in Alexandria, Virginia, on July 19, 1976; sponsored by the Carcinogenesis Research Program of the National Cancer Institute.

- Experimental Pathology Workshop, organized by Dr. D. Goodman and Dr. S. Barron, was held in Worcester, Massachusetts, on October 26-27, 1976; sponsored by the National Cancer Institute.

- Workshop on Aryl Hydrocarbon Hydroxylase and Lung Cancer, organized by Dr. D. Longfellow, was held at the National Institutes of Health in Bethesda, Maryland, on November 16-17, 1976; sponsored by the National Cancer Institute.

- Workshop on DNA Repair and Carcinogenesis, organized by Dr. D. Longfellow and Dr. T. Domanski, was held in Alexandria, Virginia, on December 8-10, 1976; sponsored by the Division of Cancer Research Resources and Centers of the National Cancer Institute.

- International Conference on Polycyclic Hydrocarbon Carcinogenesis, Chemistry, Biology, and Environment, organized by Dr. H. Gelboin, Dr. P. Ts'o (The Johns Hopkins University), and by Dr. C. Nagato (National Cancer Center Research Institute, Tokyo, Japan), was held in New Orleans, Louisiana, on January 23-26, 1977; sponsored by the National Cancer Institute, The Johns Hopkins University, and the NCCRI of Tokyo.

- Conference on Chemical Synthesis of Retinoids, organized by Dr. M. Sporn, was held at the National Institutes of Health on February 17-18, 1977; sponsored by the Carcinogenesis Research Program of the National Cancer Institute.

- Research Conference on Mechanisms of Tumor Promotion and Cocarcinogenesis, organized by cochairmen Dr. T. Slaga (Oak Ridge National Laboratories) and Dr. P. Nettesheim (Oak Ridge National Laboratories), Dr. R. Boutwell (the University of Wisconsin), Dr. R. Griesemer (formerly of Oak Ridge National Laboratories), Dr. H. Hennings, Dr. J. Selkirk, Dr. A. Sivak (Arthur D. Little, Inc.), Dr. R. Tennant, and Dr. S. Yuspa, was held in Gatlinburg, Tennessee, on March 28-31, 1977; sponsored by the U.S. Energy Research and Development Administration and the Carcinogenesis Research Program of the National Cancer Institute.

- Workshop on Carcinogenesis Studies in Human Cells and Tissues, organized by Dr. C. Harris, was held at the Given Institute of Pathobiology in Aspen, Colorado, on August 14-19, 1977; sponsored by the National Cancer Institute.

Carcinogenesis Publications

Total Number of Publications in FY 1977

Program	Total No.	Per Cent
Authorship	894	100.0%
(a) INTRAMURAL PROGRAM Exclusively intramural staff	142	15.8%
(b) COLLABORATIVE PROGRAM Exclusively contract-supported investigators	693	77.5%
(c) INTRAMURAL/COLLABORATIVE PROGRAM Joint	59	6.7%

4. FISCAL INFORMATION

The following charts and tables report the present distribution of funds in the Carcinogenesis Program (for the Transition Quarter and Fiscal Year 1977), including Direct Operations and Contracts:

FIGURE 1 - Allocations-Direct Operations and Contracts (Transitional Quarter)
FIGURE 2 - Allocations-Direct Operations and Contracts (Fiscal Year 1977)
FIGURE 3 - Distribution of Contract Funds by Operational Units
FIGURE 4 - Trend in Budget and Positions
TABLE I - Analysis of Contract Activities by Operational Units (with supporting Program Areas)
TABLE II - Analysis of Contracts by Operational Units

CARCINOGENESIS PROGRAM

Allocations - Direct Operations and Contracts
Transitional Quarter

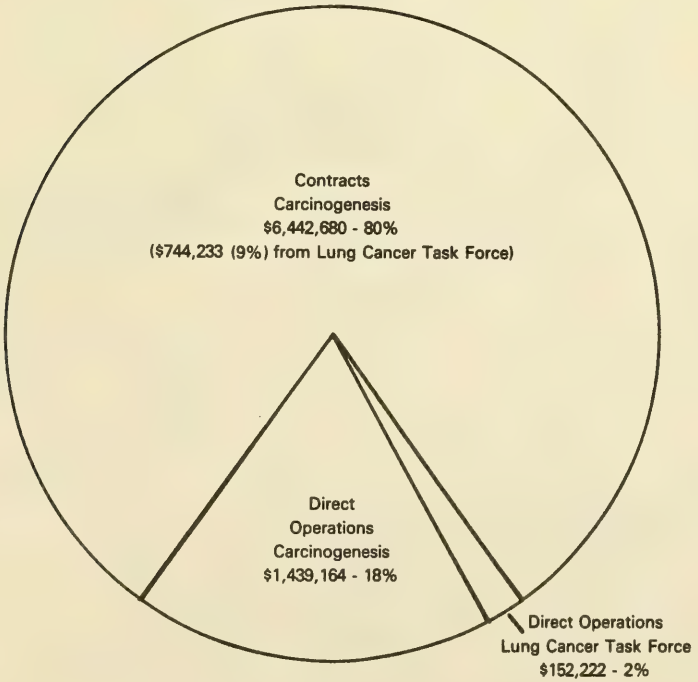


FIGURE 1

CARCINOGENESIS PROGRAM

Allocations - Direct Operations and Contracts
Fiscal Year 1977

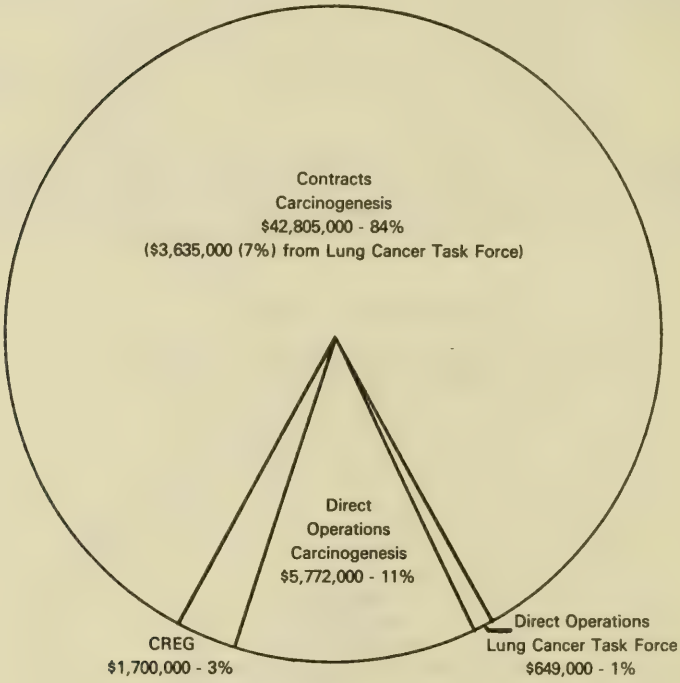
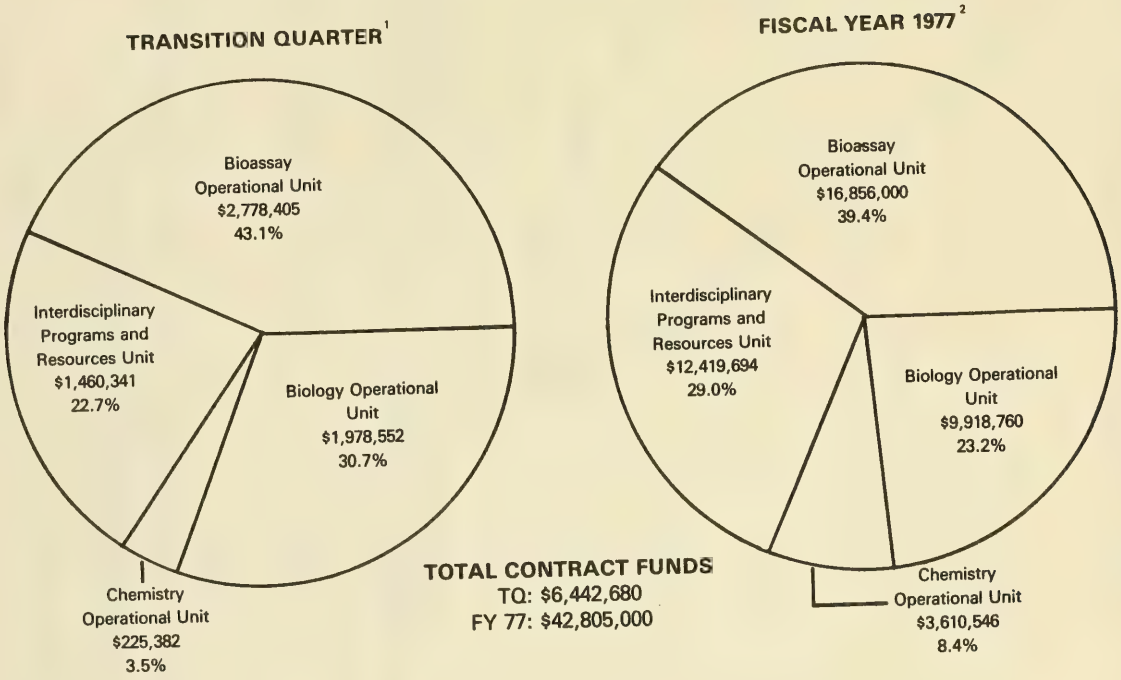


FIGURE 2

CARCINOGENESIS PROGRAM

Distribution of Contract Funds by Operational Units


TRANSITION QUARTER[1]
Bioassay Operational Unit $2,778,405 43.1%
Interdisciplinary Programs and Resources Unit $1,460,341 22.7%
Biology Operational Unit $1,978,552 30.7%
Chemistry Operational Unit $225,382 3.5%
TOTAL CONTRACT FUNDS
TQ: $6,442,680
FY 77: $42,805,000
FISCAL YEAR 1977[2]
Bioassay Operational Unit $16,856,000 39.4%
Interdisciplinary Programs and Resources Unit $12,419,694 29.0%
Biology Operational Unit $9,918,760 23.2%
Chemistry Operational Unit $3,610,546 8.4%

[1]Obligated funding information available as of September 30, 1976

[2]Obligated and estimated funding information available as of April 25, 1977

FIGURE 3

TREND IN BUDGET AND POSITIONS

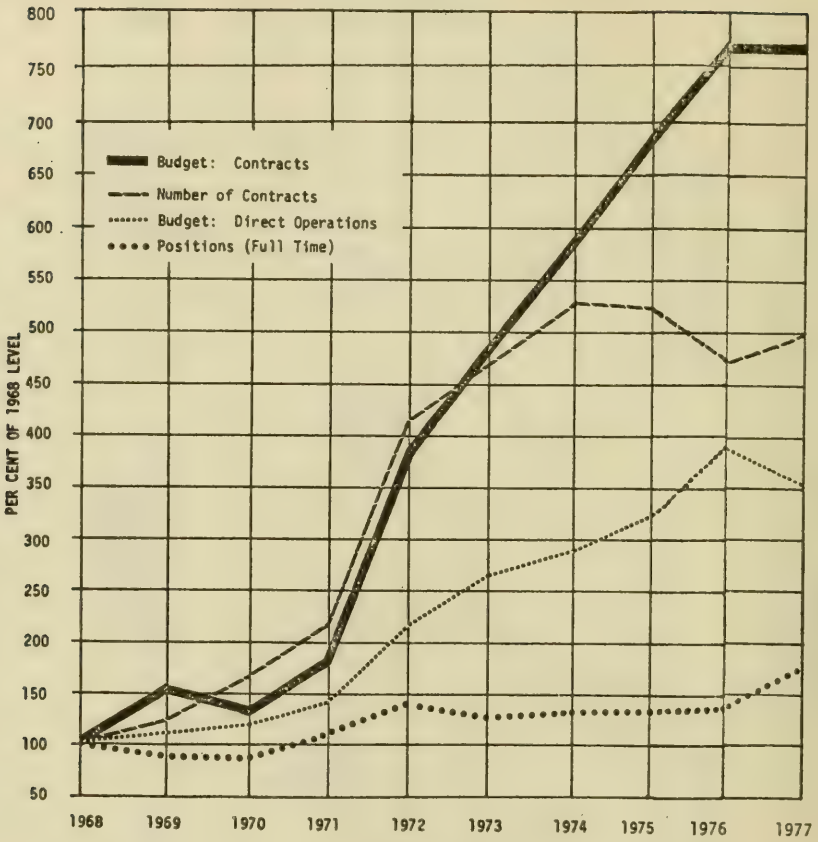


FIGURE 4

In FY 1977, 51 new positions were allocated to the Carcinogenesis Program for distribution in the following areas:

CARCINOGENESIS RESEARCH - Intramural: 18
Collaborative Program: 2

CARCINOGENESIS TESTING - (currently designated as Bioassay Operational Unit): 31

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES BY OPERATIONAL UNIT (WITH SUPPORTING PROGRAM AREAS) IN THE CARCINOGENESIS PROGRAM

OPERATIONAL UNIT	NO. OF CONTRACTS	TOTAL AMOUNT[1]	PERCENT
TOTAL →	165	$49,247,680	(100)
BIOASSAY	46	$19,634,405	39.8
Bioassay	14	10,402,377	21.1
Information	11	3,114,678	6.3
In Vitro	21	6,117,350	12.4
BIOLOGY	60	$11,897,312	24.2
Colon	9	1,306,403	2.7
Lung	16	4,379,233	8.9
Models	35	6,211,676	12.6
CHEMISTRY	33	$ 3,835,928	7.8
Chemistry & Molecular Carcinogenesis	22	2,813,855	5.7
Carcinogen Metabolism & Toxicology	11	1,022,073	2.1
INTERDISCIPLINARY PROGRAMS AND RESOURCES	26	$13,880,035	28.2
Information/Resources	15	1,523,402	3.1
Office of the Associate Director	11	12,356,633	25.1

[1]Includes Transitional Quarter and FY 1977 contract funds

TABLE II

ANALYSIS OF CONTRACTS BY OPERATIONAL UNITS
IN THE CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
1. BIOASSAY OPERATIONAL UNIT	
BIOASSAY PROGRAM	
CALIFORNIA, UNIVERSITY OF (at Davis) NO1-CP-65845	Biology of Neoplastic Liver Lesions in Mice
CHARLES RIVER BREEDING LABORATORIES NO1-CP-55651	Establishment of a Gnotobiote Originated Rodent Production Colony
CLEVELAND METROPOLITAN GENERAL HOSPITAL NO1-CP-65840	Support Mouse Colony with Liver Tumor Incidence
EXPERIMENTAL PATHOLOGY LABORATORIES, INC. NO1-CP-65731	Animal Pathology Support
HARLAN INDUSTRIES, INC. NO1-CP-65737	Establishment of a Rodent Production Colony
HOWARD UNIVERSITY NO1-CP-33266	Chemical and Biological Investigation of Potential Carcinogens from Plants
JOHNS HOPKINS UNIVERSITY NO1-CP-65768	Animal Pathology Support
MARYLAND, UNIVERSITY OF NO1-CP-65752	Biology of Neoplastic Liver Lesions in Mice
MITRE CORPORATION/METREK DIVISION NO1-CP-65863	Preparation of Carcinogen Bioassay Reports
NATIONAL ACADEMY OF SCIENCES/ INSTITUTE OF LABORATORY ANIMAL RESOURCES NO1-CP-65805	Histological Classification of Laboratory Animal Tumors

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
NCI-FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) NO1-CO-25423	Project 8 - Bioassay Research
TEMPLE UNIVERSITY NO1-CP-43271	A Study on UV Carcinogenesis
TEXAS, UNIVERSITY OF NO1-CP-65846	Biology of Neoplastic Liver Lesions in Mice
TRACOR JITCO, INC. NO1-CP-43350	Bioassay Prime Contract
IN VITRO PROGRAM	
AMERICAN HEALTH FOUNDATION NO1-CP-55705	Development of Detailed Methods and Protocols for Carcinogen Screening Using Cell Culture Assays
ARTHUR D. LITTLE, INC. NO1-CP-55711	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays
BIOLABS, INC. NO1-CP-45615	In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens
CHILDREN'S HOSPITAL OF AKRON NO1-CP-55706	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) YO1-CP-50200	NCI-ERDA Carcinogenesis Program
ILLINOIS, UNIVERSITY OF NO1-CP-23303	Temperature Sensitive Mutants in In Vitro Carcinogenesis
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER NO1-CP-55630	The Significance of Experimental Carcinogenesis Data to Man

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
INVERESK RESEARCH INTERNATIONAL NO1-CP-75858	Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens
JOHN L. SMITH MEMORIAL FOR CANCER RESEARCH, PFIZER, INC. NO1-CP-55703	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays--Task I
LEO GOODWIN INSTITUTE FOR CANCER RESEARCH NO1-CP-65804)	Production of an In-Bred Syrian Hamster Colony
LITTON BIONETICS, INC. NO1-CP-65853	Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogen Screening
LITTON BIONETICS, Inc. NO1-CP-65856	Validation and Utilization of Microbial Mutagenesis Systems as a Prescreen for Chemical Carcinogens
MICROBIOLOGICAL ASSOCIATES NO1-CP-55670	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assay
NCI-FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) NO1-CO-25423	Project - In Vitro Carcinogenesis
NEW YORK MEDICAL COLLEGE NO1-CP-65855	Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens
NORTH CAROLINA, UNIVERSITY OF NO1-CP-55707	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays
OHIO STATE UNIVERSITY NO1-CP-43276	In Vitro Study of the Nature of Interaction Between Chemical and Viral Carcinogens

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
SOUTHERN CALIFORNIA, UNIVERSITY OF NO1-CP-65831	Carcinogenesis In Vitro: Initiation and Promotion
STANFORD RESEARCH INSTITUTE NO1-CP-55701	Development of Detailed Methods and Protocols for Carcinogenic Screening Using Cell Culture Assays
STANFORD RESEARCH INSTITUTE NO1-CP-65854	Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening
STANFORD RESEARCH INSTITUTE NO1-CP-65857	Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens
INFORMATION PROGRAM	
CALIFORNIA, UNIVERSITY OF (at Davis) NO1-CP-75816	Computer Aided Prediction of Metabolites for Carcinogenicity Studies
EG&G/MASON RESEARCH INSTITUTE NO1-CP-43257	Carcinogenesis Bioassay Data System Operational Support
ENVIRO CONTROL, INC. NO1-CP-65808	Information Resources
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory Y01-CP-20203	Environmental Mutagen Information Center (EMIC)
FRANKLIN INSTITUTE RESEARCH LABORATORIES NO1-CP-43293	Carcinogenesis Abstracts
FRANKLIN INSTITUTE RESEARCH LABORATORIES NO1-CP-65810	Information Resources

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE NO1-CP-65811	Information Resources
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER NO1-CP-45608	Program for the Evaluation of Carcinogenic Risk of Chemicals to Man
STANFORD RESEARCH INSTITUTE NO1-CP-33285	A Research Program to Acquire and Analyze Information on Chemicals That Have Impact on Man and His environment
SYSTEMS SCIENCE NO1-CP-65760	Information Resources
TRACOR JITCO, INC. NO1-CP-65809	Information Resources

2. BIOLOGY OPERATIONAL UNIT

AICHI CANCER CENTER RESEARCH INSTITUTE NO1-CP-55650	Role of Hormonal Induction of Permanent Cellular Alterations during Perinatal Exposure on Carcinogenesis
ALTON OCHSNER MEDICAL FOUNDATION NO1-CP-65764	Study of the Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats
ALTON OCHSNER MEDICAL FOUNDATION NO1-CP-43229	Carcinogenesis by Radiation Plus Estrogen
AMERICAN HEALTH FOUNDATION NO1-CP-33208	Studies in Colon Carcinogenesis
AMERICAN TYPE CULTURE COLLECTION NO1-CP-65751	Development of Cell Strains from Ductal and Exocrine Portions of the Pancreas

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
BAYLOR COLLEGE OF MEDICINE NO1-CP-65837	Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract
BIOTECH RESEARCH LABORATORIES, INC. NO1-CP-55640	Criteria for In Vitro Cell Transformation by Viruses and Chemical Carcinogens
CALIFORNIA, UNIVERSITY OF (at Los Angeles) NO1-CP-33380	Fecal Flora Studies
CHICAGO, UNIVERSITY OF NO1-CP-65777	Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract
CHILDREN'S HOSPITAL OF LOS ANGELES NO1-CP-55641	Fibrinolysis as a Parameter of In Vitro Transformation
COLORADO, UNIVERSITY OF NO1-CP-75875 (Formerly - Stanford University NO1-CP-55649)	Tissue Interactions in Induction and Perpetuation of Hormonally-Induced Permanent Cellular Alterations
COLORADO, UNIVERSITY OF NO1-CP-65849	In Vitro Cultivation of Normal, Epithelial, Human Prostatic Cells
DARTMOUTH COLLEGE NO1-CP-65776	Development of Animal for Oat Cell Carcinoma of the Lung
DARTMOUTH COLLEGE NO1-CP-55708	Chemical and Structural Requirements for Pancreas Uptake and Excretion: Rats and Hamsters
DARTMOUTH COLLEGE NO1-CP-33378	Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats
ERDA-NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) YO1-CP-60219	Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
ERDA-NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) Y01-CP-30213	Synergistic Interaction of Hormones and Neutron Radiation of Mammary Gland Carcinogenesis
EXPERIMENTAL PATHOLOGY LABORATORIES, INC. N01-CP-65763	Resource for Microscopic and Autoradiographic Technology
GEORGIA, MEDICAL COLLEGE OF N01-CP-55656	Study of the Potential for Metabolic Activation in Animal Pancreas
GEORGIA, MEDICAL COLLEGE OF N01-CP-43282	Epidemiological Studies of the Incidence of Colon Cancer among Blacks and Whites
HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH N01-CP-33273	Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens
HAZELTON LABORATOIRIES, INC. N01-CP-23287	Induction of Adenocarcinoma of the Prostate
IIT RESEARCH INSTITUTE N01-CP-43289	Supply of Animals Treated with Epithelial Carcinogens
IIT RESEARCH INSTITUTE N01-CP-23292	Studies of Modulating Factors in Epithelial Carcinogenesis
ILLNOIS, UNIVERSITY OF N01-CP-65843 (Formerly - Case Western Reserve University N01-CP-23284)	Maintenance and Scheduled Sacrifice of Guinea Pigs
INDIANA UNIVERSITY N01-CP-55654	Chemical and Structural Requirements for Pancreas Uptake and Excretion: Rats and Guinea Pigs
KAISER FOUNDATION RESEARCH INSTITUTE N01-CP-33215	Epidemiologic Study of Colorectal Cancer in Blacks

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
LITTON BIONETICS, INC. NO1-CP-65847	Resource for Microscopic and Autoradiographic Technology
LITTON BIONETICS, INC. NO1-CP-43274	Studies of Carcinogenesis in Human Bronchial Tissues
MARYLAND, UNIVERSITY OF NO1-CP-43237	Studies on Carcinogenesis in Human Tissues: Bronchial Epithelium, Pancreas, Breast and Colon
MAYO FOUNDATION NO1-CP-55660	Reflux as a Mechanism for Induction of Adenocarcinoma of the Pancreas
MELOY LABORATORIES, INC. NO1-CP-55613	Transplacental Carcinogenesis in the Old World Monkey, Erythrocebus Patas
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES NO1-CP-43366	Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant and Malignant Colonic Tissues in Familial Polyposis
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES NO1-CP-43232	Identification of Site of Origin in Human Pancreatic Adenocarcinoma
MICHIGAN, UNIVERSITY OF NO1-CP-55720	Organ Culture of the Prostate: A Biological Model
MICROBIOLOGICAL ASSOCIATES, INC. NO1-CP-02199	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities
MINNESOTA, UNIVERSITY OF NO1-CP-55702	Rhythmometry on Japanese and North American Female Volunteers of Different Age Groups
MINNESOTA, UNIVERSITY OF NO1-CP-33364	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract
MISSOURI, UNIVERSITY OF NO1-CP-33335	Effect of a High-Meat Diet on the Bacterial Flora and Chemical Components of Feces

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
NEBRASKA, UNIVERSITY OF N01-CP-33289	Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture
NEW YORK UNIVERSITY N01-CP-33260	Studies in Pulmonary Carcinogenesis
NORTHWESTERN UNIVERSITY N01-CP-75876 (Formerly - University of Kansas - N01-CP-23271)	Histogenesis of Guinea Pig Pancreatic Adenocarcinoma
ORGANIZATION FOR HEALTH RESEARCH (TNO) N01-CP-33330	Synergistic Interaction of Hormones and Neutron Radiation of Mammary Gland Carcinogenesis
OXFORD, UNIVERSITY OF N01-CP-23215	Procurement of Data for Determination of Disease Linkage
PASADENA FOUNDATION FOR MEDICAL RESEARCH N01-CP-65850	Primary Culture of Normal, Human Prostatic Epithelial Cells
SOUTHERN RESEARCH INSTITUTE N01-CP-22064	Organ Culture Assay of Vitamin A Analogs
SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION N01-CP-33379	Studies of Hormonal Factors of the Human and Animal Prostate
STATE UNIVERSITY OF NEW YORK (at Stony Brook) N01-CP-33361	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi
STOCKHOLM, UNIVERSITY OF N01-CP-33363	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract
TENNESSEE, UNIVERSITY OF N01-CP-33369	Maintenance of Organ Explants from Rodent Pancreas
TEXAS, UNIVERSITY OF N01-CP-33362	Studies of Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
TORONTO, UNIVERSITY OF NO1-CP-75879	Biochemical and Morphologic Components of Hepatic Carcinogenesis
UTAH, UNIVERSITY OF NO1-CP-55709	Quantitation of Physiological Reflux in Pancreatic Duct of Primates
VERMONT, UNIVERSITY OF NO1-CP-33360	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi
VETERANS ADMINISTRATION HOSPITAL (Tampa, Florida) YO1-CP-55625	Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis
VETERANS ADMINISTRATION HOSPITAL (Washington, D.C.) YO1-CP-60204	Studies on Normal, Premalignant and Malignant Respiratory Epithelium of Humans
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY NO1-CP-55685	Relationship of Fecal Neutral Sterols to Large Bowel Cancer Risk
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY NO1-CP-33334	Comparative Fecal Flora Studies
W. ALTON JONES CELL SCIENCE CENTER NO1-CP-65762	In Vitro Cultivation of Normal, Prostatic Epithelial Cells
WEST VIRGINIA UNIVERSITY NO1-CP-75887	Prostate Transplant Model: A System for Studying Neoplasia
WEST VIRGINIA UNIVERSITY NO1-CP-43230	Relationship of Pituitary Hormones and Androgens on Prostate Gland Metabolism

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
3. CHEMISTRY OPERATIONAL UNIT	
ALBERT EINSTEIN COLLEGE OF MEDICINE NO1-CP-55606	Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans
AMERICAN HEALTH FOUNDATION NO1-CP-55639	Metabolism of Carcinogenic Compounds
BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION NO1-CP-44337	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment
CALIFORNIA, UNIVERSITY OF (at San Diego) NO1-CP-33232	Study on Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay
CALIFORNIA, UNIVERSITY OF (at San Francisco) NO1-CP-43239	Selection and Propagation of Somatic Cells Having Specific Physiological Mutations
ERDA/NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) YO1-CP-50202	Repair Mechanisms in Carcinogenesis
ERDA/NCI INTERAGENCY AGREEMENT (University of California at Berkeley) YO1-CP-50203	Molecular Processes Involved in the Carcinogenic Action of Polycylic Aromatic Hydrocarbons
FRED HUTCHINSON CANCER RESEARCH CENTER OF PACIFIC NORTHWEST RESEARCH NO1-CP-55719	Metabolism of Carcinogenic Compounds
HEALTH RESEARCH, INC./ ROSWELL MEMORIAL INSTITUTE NO1-CP-55629	Aryl Hydrocarbon Hydroxylase in Human Lymphocytes and the Relationship to Chemical Carcinogenesis

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
HEBREW UNIVERSITY NO1-CP-43307	Mammalian Cell Transport Systems
INSTITUTE OF CANCER RESEARCH/ CHESTER BEATTY RESEARCH INSTITUTE NO1-CP-33367	Nature of the Polycyclic Hydrocarbon-Nucleic Acid Compound in Hydrocarbon Carcinogenesis
JOHNS HOPKINS UNIVERSITY NO1-CP-55713	The Significance to Mutagenesis in Carcinogenesis
JOHNS HOPKINS UNIVERSITY NO1-CP-45610	Studies of Mammalian Cell Transport Systems
MANITOBA, UNIVERSITY OF NO1-CP-43250	Isolation, Purification, and Characterization of Human Prolactin
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-43265	Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-33238	Interactions Between Diet and Chemical Carcinogenesis: A Bioassay System
MASSACHUSETTS INSTITUTE OF TECHNOLOGY NO1-CP-33315	Environmental Occurrence of N-Nitroso Compounds
MICROBIOLOGICAL ASSOCIATES NO1-CP-55605	Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans
MISSISSIPPI, UNIVERSITY OF NO1-CP-43347	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment
NEW YORK UNIVERSITY MEDICAL CENTER NO1-CP-33279	Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
OHIO STATE UNIVERSITY NO1-CP-43248	Preparation of Polymers Containing Carcinogenic Moieties
ONTARIO CANCER INSTITUTE NO1-CP-43331	Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions
OREGON STATE UNIVERSITY NO1-CP-43348	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment
POLYSCIENCES, INC. NO1-CP-23245	Optimizing Electrophoretic Separation of Proteins and Nucleic Acids with New Hydrogels
ROCHESTER, UNIVERSITY OF NO1-CP-45611	Mammalian Cell Transport Systems
SOUTHERN RESEARCH INSTITUTE NO1-CP-55721	Metabolism of Carcinogenic Compounds
TEXAS, UNIVERSITY OF (at Houston) NO1-CP-55604	Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans
THERMO ELECTRON CORPORATION NO1-CP-45623	Development and Application of Methods for Analysis of N-Nitroso Compounds and Their Precursors in the Environment
VANDERBILT UNIVERSITY NO1-CP-65730	Non-Histone, DNA-Binding Proteins in Chemical Carcinogenesis
WEIZMANN INSTITUTE OF SCIENCE NO1-CP-02217	Study of the Role of Enzyme Induction in Chemical Carcinogenesis
WEIZMANN INSTITUTE OF SCIENCE NO1-CP-33220	Studies of Alterations Produced by Chemical Carcinogens in Viral and Cellular Gene Expressions

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
WISCONSIN CLINICAL CANCER CENTER/UNIVERSITY OF WISCONSIN NO1-CP-65734	Induction of Aryl Hydrocarbon Hydroxylase in Cultured Human Lymphocytes
WISTAR INSTITUTE NO1-CP-55655	Significance of Mutagenesis in Carcinogenesis
YALE UNIVERSITY NO1-CP-55673	Selection and Propagation of Monosomic and Haploid Cell Lines

4. INTERDISCIPLINARY PROGRAMS AND RESOURCES OPERATIONAL UNIT

CALIFORNIA, UNIVERSITY OF (at San Diego) NO1-CP-33403	Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogens
CHICAGO, UNIVERSITY OF NO1-CP-33385	Synthesis of Derivatives of Carcinogenic Polycyclic Hydrocarbons
THE CHILDREN'S HOSPITAL OF PHILADELPHIA NO1-CP-65803	Oncogenesis and Other Late Effects of Cancer Therapy
COLUMBIA UNIVERSITY NO1-CP-23234	Analysis of the Mechanism of Chemical Carcinogenesis in Epithelial Cell Cultures
ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) YO1-CP-50200	NCI-ERDA Carcinogenesis Program
HARLAN INDUSTRIES, INC. NO1-CP-55647	Development of Colonies of Aged Rats
IIT RESEARCH INSTITUTE NO1-CP-65745	Preparation of Carcinogens
IIT RESEARCH INSTITUTE NO1-CP-55646	Chemical Repository

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
IIT RESEARCH INSTITUTE NO1-CP-33296	Production and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis
MEDIZINISCHE HOCHSCHULE HANNOVER NO1-CP-12148	Development of a Lung Tumor Model with Large European Hamsters (Cricetus cricetus L.)
MIDWEST RESEARCH INSTITUTE NO1-CP-65739	Preparation of Carcinogens
MIDWEST RESEARCH INSTITUTE NO1-CP-23270	Analytical Chemistry Resource
MIDWEST RESEARCH INSTITUTE NO1-CP-33387	Synthesis of Polycyclic Hydrocarbon Derivatives
MIDWEST RESEARCH INSTITUTE NO1-CP-23270 (Sub-Contract)	Development of a Selective Method for the Trace Analysis of Hydroxamic Acids
NCI FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) NO1-CO-25423	Operation and Maintenance of the Frederick Cancer Research Center
NEBRASKA, UNIVERSITY OF (Eppley Institute for Research in Cancer) NO1-CP-33278	A Resource for Carcinogenesis Bioassays and Releated Research
NEW HAMPSHIRE, UNIVERSITY OF NO1-CP-55675	Preparation of Various N-Nitroso Compounds
SALK INSTITUTE NO1-CP-33405	Carcinogenesis and Control of the Cell Cycle
SOUTHERN RESEARCH INSTITUTE NO1-CP-65740	Preparation of Carcinogens
STANFORD RESEARCH INSTITUTE NO1-CP-65741	Preparation of Carcinogens

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

UNIT CONTRACT	TITLE
STANFORD RESEARCH INSTITUTE NO1-CP-55672	Preparation of Various N-Nitroso Compounds
THERMO ELECTRON CORPORATION NO1-CP-65822	Production and Delivery of Three HPLC Interface Systems
UTAH, UNIVERSITY OF NO1-CP-45600	Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis

5. PROGRAM SUMMARY REPORTS

SUMMARY REPORT

BIOLOGY BRANCH

July 1, 1976 through September 30, 1977

"Plans, develops, and conducts a research program aimed at (1) elucidation of the role of chemical, physical and biological agents in the process of carcinogenesis, (2) characterization of cellular alterations associated with carcinogenesis, (3) evaluation of the relationships between mutagenesis and carcinogenesis, and (4) the development and definition of in vitro cellular transformation systems for the rapid detection of the carcinogenic actions of chemicals."

The Biology Branch has continued to develop mammalian cell in vitro carcinogenesis models for the rapid identification of cancer causing agents relevent to the human environment, and the study of events and mechanisms that accompany and/or cause mallignancy. Methods to prevent and reverse the carcinogenic process are also being investigated. Three aspects of the carcinogenic process are under study--(1) transformation of normal to neoplastic cells, (2) immunobiologic evaluation of the neoplastic state, and (3) relationship of mutational events to the transformation. The major research projects in the Biology Branch continue to be--(1) development of short-term and reliable methods, based on in vitro cell transformation, to identify carcinogenic chemicals, (2) development of methods to identify and characterize the stages of carcinogenesis, and (3) assessment of the relationship of somatic cell alterations to the neoplastic state. Responsibility for these projects was divided between two sections: Somatic Cell Genetics Section and Tumor Biology Section. Results of studies from the individual sections were coordinated at the branch level to determine their applicability to the goals of the National Cancer Program. In the Somatic Cell Genetics Section, studies concerning the modulation of transformation frequencies by sequential treatment with different agents have shown that the transformation frequency can be increased or decreased depending upon the agents used as well as the sequence in which they are applied. This is particularly important since combinations of various agents may be responsible for an increase of cancer in human populations. Conflicting studies from a number of laboratories exist concerning the role of caffeine on the frequency of mutations; current studies employing quantitative techniques have shown that caffeine interferes significantly with DNA repair but does not alter carcinogen induced mutation frequencies of Chinese hamster cells. Members of the Tumor Biology Section have continued to study specific stages in the carcinogenic process identifiable during chemical carcinogen induced neoplastic transformation of guinea pig cells in culture in order to provide valuable insights into the events that occur during development of a cancer cell. Guinea pig cells are resistant to lymphotoxin during various stages of carcinogenesis prior to expression of tumor growth; susceptibility, the inhibition of tumor cell growth, develops in close proximity or concomitantly with neoplastic transformation.

Somatic Cell Genetics Section - "*Studies (1) carcinogenesis in mammalian cells in vitro and in host-mediated in vivo and in vitro models, (2) interactions of chemical, physical and biological agents resulting in the initiation, enhancement or inhibition of neoplastic transformation, (3) effects of potential environmental carcinogens in cells in culture and in intact animals, and (4) relationships between mutagenesis and carcinogenesis in in vitro mammalian cell models and in intact animals.*" The prevention of cancer in humans depends to a large extent on finding and removing potentially harmful environmental agents. The use of mammalian cells in culture is particularly appropriate because they represent an extension of experimental animal models. The conversion of control mammalian cells to the neoplastic state in culture can be validated by injecting the cells into animals to produce progressively growing tumors which kill the animals.

The cell culture systems have been shown to be useful for studying the nature of the interaction between chemical and target and for seeking ways to modify or prevent transformation. Studies have also been carried out with combinations of agents of various classes because the combination of different agents may act synergistically or antagonistically in the production of experimental cancer; the same effects may occur in humans. X-irradiation and methyl methanesulfonate (MMS) are capable of enhancing transformation associated with a number of chemical carcinogens without altering cell cycle or the number of cells that synthesize DNA. The transitory effect of irradiation or alkylating chemicals and the lack of transformation with irradiation alone argued against the selection of a special radiation sensitive cell. Maximum enhancement occurred when the two treatments were separated by 48 hrs. The dose-response relationship with chemical carcinogen alone is proportional to carcinogen dose. The shape of the curve is consistent with transformation that is due to the interaction of a single hit rather than a large number of events because the increase in the transformations was a Poisson distribution. The dose-response relationship of transformation of Syrian hamster cells by chemical carcinogen was further investigated with and without pretreatment with X-irradiation. Different concentrations of chemical carcinogens were used with and without a constant amount of radiation. These studies again indicate that transformation is due to a "one-hit" phenomenon. Among the various experiments, the points deviate from the curve at different concentrations. A greater variation in transformation occurs among dishes with larger doses of chemical carcinogen. Nevertheless, the two curves, with and without X-irradiation, can be described by one number since the ratio of the two ➤is 8-9-fold. These results also indicate binominal variation which is a reflection of the number of cells at risk.

The cause of the transitory changes in cells by radiation or a radiomimetric agent that results in an enhancement of transformation associated with known chemical carcinogens is unknown. Radiation is known to produce single-strand DNA breaks that are repaired rapidly. It is possible, however, that some unrepaired breaks occur and are responsible for cell death; by the same token, secondary effects of radiation may require longer to occur and be responsible for mispairing which would be more vulnerable to additional insult by the addition of chemical carcinogens. Since many chemical carcinogens induce DNA damage, as demonstrated by sedimentation in alkaline sucrose, and unscheduled repair, as indicated by radioautography, the response to two chemicals, MMS

and N-acetoxy-2-fluorenylacetamide (AcAAF), was examined at different intervals using arginine-free medium in order to stop DNA synthesis and cell replication. The results of the combination treatment were compared to those obtained with MMS and AcAAF treatment alone. At 24 hrs after MMS, the two treatments result in repair which is less than that obtained by the individual treatments. At 48 hrs, the two-treatment combination is equal to that obtained by either treatment. At 72 hrs, results obtained with sequential treatments are much greater than those from either treatment alone. The synergism in repair obtained at 72 hrs may be due to less metabolic activity occurring under the inhibitory conditions imposed by arginine-free medium. With the experimental conditions for transformation, repair may be more rapid and perhaps coincide with the time of maximal transformation enhancement. This hypothesis is consistent with the concept that DNA is the primary target of a carcinogen.

The enhancement of DNA viral transformation frequency that occurs with chemical carcinogens may result from an increased integration of viral genome into cellular DNA. Simian adenovirus (SA7) DNA, associated with single-strand cell DNA, was determined by cRNA-DNA hybridization after centrifugation in alkaline glycerol gradients and viral DNA in chromosomes by in situ hybridization. Four days after MMS or 3 days after $NiSO_4$ treatment, a 12- and a 14-fold increase, respectively, in viral genome content occurred relative to SA7 alone. In the absence of scheduled DNA synthesis, SA7 genome content increased by 4-5 times during the first 24 hrs when virus was added immediately after MMS treatment; no increase occurred when virus was added 24 hrs after MMS. Individually transformed colonies resulting from MMS-SA7 alone had similar numbers of SA7 genome/cell. SA7 DNA appears to be inserted randomly among all chromosomes and nuclei and the number of grains varies from cell to cell within a colony. The increased incorporation of SA7 DNA observed shortly after treatment may be due to an increased uptake of viral DNA on a per culture rather than a per cell basis. Alternatively the SA7 DNA may be progressively removed during foci growth.

A relatively new chromosome technique is being evaluated for its ability to rapidly identify potential carcinogens. Sister chromatid exchanges (SCE), the interchange of DNA between replication products and apparently homologous loci, can now be visualized by staining procedures on cytological chromosome preparations. Although some chemicals increased the frequency of chromosome aberrations, no correlation was found with SCE frequency. SCE are induced at high frequencies by chemicals at concentrations that fail to produce a significant increase in chromosome aberrations, further emphasizing the sensitivity of this method. A larger number of physical and chemical agents must be tested in conjunction with methylated oxypurine treatment to establish the factors affecting the SCE formation. If SCE induction is a reflection of mutagenesis or carcinogenesis, SCE measurements on human or mammalian systems should become a necessary test within a complex program for identification of environmental mutagens or carcinogens.

During the past year, three reviews have been written dealing with chemical carcinogenesis. In two, the relationship of chromosome changes to neoplastic cell transformation was put into perspective . The conclusions can be summarized in six statements: (1) Addition of a carcinogen to mammalian cells usually causes an immediate effect on the chromosomes resulting in a series

of events which lead to rearrangements of chromatid material; (2) The immediate effect of a carcinogen on the chromosomes of mammalian cells may be random or nonrandom and probably reflects species genomic properties; (3) The correlation between lethality and the frequency of chromosome and chromatid aberrations suggests that the genetic alterations may be responsible for reproductive death; (4) No universal alteration in specific numbers or visible structure is associated with chemical or viral transformation. In fact, transformation may occur without any visible chromosome alteration; (5) Chromosome variations are independent of the etiologic agent. Different carcinogens may produce transformation associated with the same specific marker, but not all transformed lines have the same markers even with the same chemical, and (6) Chromosome deviation in cancer is probably species-dependent and contributes to the characteristics of malignancy. The presence of identical banding patterns in transformed lines and tumor-derived cultures indicates that the transformed cells produce tumors. The existing in vitro systems utilizing mammalian cells for the study of carcinogenesis were reviewed. It is important to realize that such in vitro systems correlate well with in vivo results and that in vitro results actually parallel the in vivo experiments. One of the major obstacles to the development of in vitro techniques for finding potential chemical carcinogens is that although many morphologic and behavioral alterations generally accompany transformation, no universal criterion exists for the identification of cell transformation in vitro. The most significant characteristic to date, since chemically transformed cells do not have highly specific common antigen markers, is malignancy (the ability to produce tumors), followed by the ability to form colonies in semi-solid medium. The results with epithelial-like cells are confusing and will remain so until transformations that result in indisputable carcinomas with a relatively high efficiency are obtained. Two encouraging approaches which have been neglected for obtaining transformation are organ culture and the host-mediated methods. Several mechanisms have been proposed for the initial events leading to neoplastic conversion of normal cells. The close association between the mutagenic and carcinogenic activities of a wide variety of chemical carcinogens has focused attention on the interaction between chemical carcinogens and cellular DNA. However, the frequency of transformation in mammalian cells is much higher than that reported for mutagenesis with the same chemical. DNA is the critical site for a mutagen, but the critical target of a chemical carcinogen is unknown.

Investigation of the mutagenesis of cultured mammalian cells and the relationship of mutagenesis to malignant transformation have been continued by studies of factors and conditions necessary for reliable measurements of induced mutation frequencies in different cell types. Diploid human cells from normal and Xeroderma pigmentosum patients are mutable by various carcinogens, but many factors affect the observable number of mutants. An assay which uses thioguanine resistance as the genetic marker is currently under study with respect to linearity, efficiency of recovery, and maximum expression of the new phenotype. Also, the effects of caffeine on mutagen-treated Chinese hamster V79 cells have been studied. Platinum diamine dichloro-cis, an antitumor agent used in humans, induces mutation thioguanine resistance and a very high frequency of sister chromatid exchange. Caffeine has little, if any, effect on these actions. Similarly, AcAAF induces mutants in V79 cells by

a mechanism not influenced by caffeine up to 1 mM. Both enhancement and inhibition of mutagenesis had been reported and these conflicting results are now shown to be artifacts of non-quantitative mutagenesis assays. In contrast to the mutation results, caffeine greatly potentiates the toxicity of AcAAF and the platinum compound, indicating that it interferes significantly with the process of DNA repair. Survival studies have been severely hampered by extreme variability, 100-fold and greater, in this and other laboratories, despite repeatable mutation frequencies. In order to relate mutation to changes in DNA repair activity, the variability of survival was studied in detail. It was found necessary to strictly control cell growth conditions, procedures for mixing chemical and medium, and the amount of chemical available per cell. Caffeine is rapidly metabolized and AcAAF is highly reactive with medium components, so it is necessary to determine survival under chemical-to-cell conditions identical to those existing during mutagenesis. Large numbers of cells are treated for mutation studies and, therefore, the associated survival must be determined in the presence of large numbers of non-proliferating V79 cells (X-irradiated) that maintain their metabolic activity over the treatment period. Under these conditions, cell survival to combinations of mutagen and caffeine is repeatable and relatable to the frequency of mutation.

Tumor Biology Section - "*Studies somatic cell alterations associated with the transition from the growth controlled to the neoplastic state to define intracellular and extracellular events at the cellular and host levels essential for the development, maintenance and/or suppression of neoplasia.*" Investigations have continued to explore the stages in the development of neoplasia utilizing mammalian cell culture models of carcinogenesis. The model system using guinea pig cells developed in the Biology Branch during the past five years is a valuable resource as discrete stages in the neoplastic transformation process persist for extended periods of time. This allows introspection into the events associated with carcinogenesis at the various stages prior to, at the time of, and following neoplastic transformation.

Previous studies demonstrated that chemical carcinogen induced morphologically transformed and neoplastic cells but not nontumor producing cells were susceptible as measured by inhibition of cell colony growth, to soluble factor(s) produced by cultured leukocytes from healthy nonimmune syngeneic animals. The soluble colony inhibitory activity is due to lymphotoxin, one or more of a number of lymphokines produced by lymphoid cells. Current investigations have revealed that lymphotoxin susceptibility develops in close proximity to neoplastic transformation. At the stages of carcinogenesis prior to neoplastic transformation cells are resistant to lymphotoxin. Descendents of the same populations of lymphotoxin resistant cells once they subsequently develop neoplastic transformation as indicated by their ability to produce tumors when inoculated into a host animal are susceptible to the cytotoxic activity of lymphotoxin. Once acquired lymphotoxin susceptibility is a stable property from one cell generation to the next with each different tumor producing cell culture possessing a quantitatively characteristic degree of lymphotoxin susceptibility. Continuing evaluations are aimed at defining whether lymphotoxin susceptibility is a fundamental property of the neoplastic state and the extent to which lymphotoxin susceptibility participates in the ability of the cell to grow as a tumor in the host.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04575-03 BGY

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Relationships Between In Vitro Mutagenesis and Chemical Carcinogenesis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. C. Myhr	Senior Staff Fellow	BGY NCI
OTHER:	J. A. DiPaolo	Chief, Biology Branch	BGY NCI
	D. Turnbull	Visiting Fellow	BGY NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biology Branch, Carcinogenesis Program

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to determine whether mutational processes are involved in in vitro malignant transformation of mammalian cells by diverse chemical and physical carcinogens. Research efforts during the past year have centered on (1) determination of cell culture conditions necessary to maintain morphological differences between transformed and normal cells, (2) standardization of the survival responses of Chinese hamster V79 cells to chemical carcinogens, caffeine, and UV light, and (3) study of assay conditions necessary for quantitative measurements of induced mutation frequencies in cultured X. pigmentosum human cells. Future efforts will be directed toward simultaneous mutagenesis and transformation assays utilizing both human and Syrian hamster embryo cells in culture.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The overall objective of this project is to determine whether mutational processes are involved in the neoplastic transformation of mammalian cells by diverse chemical and physical mutagens. The accomplishments of this goal depends on quantitative assays for mutant frequencies and the ability to perform both mutagenesis and carcinogenesis assays with the same type of cultured cells. Accordingly, the current objectives are:

1. To establish survival curves for Chinese hamster V79 cells exposed to combinations of N-acetoxy-2-fluorenylacetamide (AcAAF) and caffeine under conditions of previous mutagenesis studies.

2. To establish assay conditions for quantitative mutagenesis studies with X. pigmentosum and other human cells in culture.

3. To examine the mutagenic potential of the cancer chemotherapeutic agent cis platinum diamine dichloride (NCS 119875) alone and in combination with caffeine.

Methods Employed: Standard cell culture procedures were used in determining cloning efficiencies and survival to toxic treatments as measured by colony-forming ability. Morphologic observations were performed by phase microscopy. Mutagenesis assays employed the thioguanine-resistance marker and were carried out according to procedures developed by this laboratory. Previous studies were greatly distorted by crossfeeding between mutants and non-mutants in treated colonies of cells. For quantitative studies, cells are reseeded at various times following mutagenic treatment to permit maximum expression of the induced mutant phenotype before selection of the mutants without interference from metabolic crossfeeding by wild-type cells. The attainment of maximum induced mutation frequencies is essential for the determination of dose-response relationships and comparisons of mutagenic efficiencies of different carcinogens. Also, the effects of DNA repair-modifying agents and studies of repair-deficient cells must be related to the maximum attainable mutation frequencies. Further assay requirements are being found necessary to account for the metabolic activities of non-malignant human cells in culture, as opposed to the Chinese hamster V79 cell line.

Major Findings: 1. AcAAF induces thioquanine-resistant mutants in Chinese hamster V79 cells in a highly repeatable, dose - dependent manner. Further treatment with caffeine potentiates the toxicity of a given dose of AcAAF but has no effect on the frequency of induced mutants per survivors. The effect of caffeine has been in dispute, since both enhancement and inhibition of mutagenesis have been reported. This study has shown that the conflicting earlier studies were artifacts of non-quantitative mutagenesis assays and were not caused by differences in the concentrations of caffeine used.

2. In contrast to the mutation results, the survival of cells to treatments of AcAAF and caffeine combinations was extremely variable. Other laboratories had similar problems,reporting 100-fold and greater differences in survival

for given doses of carcinogen plus caffeine. In order to determine adequately the survival-mutagenesis relationships, it was necessary, therefore, to investigate the factors controlling the toxicity of treatment. Variability of cell response was found related to variability in the morphologic states of the cell cultures and strict control of medium components became necessary. Treatment procedures were altered to reduce AcAAF decomposition prior to exposure to the cells, improve the mixing of chemical with medium in culture dishes, and reduce temperature and pH variations during the treatment period. It was further shown that survival must be determined under the same chemical-to-cell ratios that exist during mutagenesis, since caffeine is rapidly metabolized and AcAAF is highly reactive. Because large numbers of cells are necessary in mutagenesis experiments, the associated survival must be determined in the presence of non-proliferating V79 cells (X-irradiated) that maintain their metabolic activity over the treatment period. Under these conditions, cell survival to combinations of AcAAF and caffeine is repeatable and is relatable to the frequency of mutation.

3. Mutagenesis of diploid human cells in culture is complicated by low cloning efficiencies and the propensity of these cells to engage in metabolic crossfeeding through many cell-to-cell contacts. Preliminary results show that published procedures do not adequately account for these problems. The linearity of the assay with respect to the number of resistant colonies (presumptive mutants) per culture vessel is not known nor has the effect of reseeding of treated cells on the maximum induced frequency of mutants been investigated. These studies are now in progress for normal and Xeroderma pigmentosum human cells.

4. cis Platinum diamine dichloride is an active antitumor agent in animals and man. Published reports show that it binds to DNA and causes chromosome aberrations. The compound also induces mutation to thioguanine resistance and induces a very high frequency of sister chromatid exchanges (SCE) in V79 cells. The frequencies of induced mutations and SCE do not appear to be significantly affected by post-treatment with caffeine (0.75 mM). This is another example of the lack of influence by caffeine on the error proneness of DNA repair in a rodent cell line.

Significance to Biomedical Research and the Program of the Institute: Mutagenesis has long been a suspected, but undemonstrated, mechanism for chemical and physical carcinogenesis. Since 50-90% of human cancers have been estimated to be caused by environmental agents, it becomes extremely important to understand the principal mechanisms involved. The ability to perform, simultaneously, mutation and malignant transformation assays with a cell system exposed to mutagens/carcinogens is a crucial step toward understanding the mode of action of these chemicals.

Proposed Course of the Project: The Chinese hamster V79 cell line will continue to be used as a model for studies of the mutation process and for developing quantitative assays for resistance to ouabain. The mutagenic activities of several activated carcinogens will be determined. Mutagenesis of human cells will also be further explored in order to study the involvement

of DNA repair processes that probably do not exist in rodent cell lines or primary cultures. Thioguanine-resistance and ouabain-resistance are the genetic markers that will be used. When stable culture conditions for Syrian hamster embryo cells are re-established, a concerted effort will be made to relate mutagenesis to malignant transformation of these cells. It is expected that transformed lines of Syrian hamster cells, because of their higher cloning efficiency, will be used to help establish the mutation assay conditions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04629-12 BGY

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Mechanism of Cell Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. J. Gart	Head, Mathematical Statistics and Applied Mathematical Section	B NCI
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COOPERATING UNITS (if any)
Mathematical Statistics and Applied Mathematical Section, B, DCCP

LAB/BRANCH
Biology Branch, Carcinogenesis Program

SECTION
Somatic Cell Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The conditions which are responsible for or can alter in vitro transformation will be investigated further, with specific emphasis on the use of UV, X-rays, combinations of carcinogens of different classes, the combination of in vivo-in vitro systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. Studies on the mechanisms of early events of carcinogen-cell interaction will be continued, as well as investigation of different proximate carcinogens and metabolic inhibitors. Special emphasis will be given to the requirements necessary to obtain neoplastic transformation of human fetal cells. The testing for the tumorigenicity of cells exposed to different agents for different time intervals will continue.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The primary objective of this project is to establish conditions and methods for in vitro quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.

2. Define the required conditions for the transformation of human fetal cells.

3. Determination of ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.

4. Development of assays to determine whether in vitro transformation is accompanied by the appearance of tumor specific antigens.

5. Determination of the role of nononcogenic and oncogenic viruses in chemical transformation.

6. Determination of the ultrastructural changes of cells during the course of in vitro chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

7. Determination of the role of radiation and alkylating compounds in enhancing neoplastic transformation by chemicals.

8. The transformation of epithelial-like cells which result in the formation of carcinomas when the cells are transplanted into animals.

9. Analysis of variations in populations of somatic cells and their correlation with genetic changes.

Methods Employed: All procedures performed are with the view of quantitating phenomena in vitro. Such procedures are required to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with in vitro transformation. Cells used come from freshly isolated cells from animals and humans that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with nontransformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole embryos or may be from specific organs. The cells are exposed to chemical carcinogen transplacentally or prior to or subsequent to seeding the cells in Petri dishes.

Approximately one week subsequent to treatment, the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity, and spectrum of morphology of both the normal and transformed colonies. The

frequency of transformation is expressed in a number of different ways. These take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

Major Findings: During this past reporting period, three reviews which are summarized in two paragraphs, were written on chemical carcinogenesis and chromosome changes in carcinogen transformed mammalian cells. In addition to an analysis of the existing in vitro systems with mammalian cells for studying carcinogenesis, an attempt was made to put into perspective the significance and definitions of transformation, interactions involving diverse agents and the significance of carcinogen-DNA interactions. It is apparent that many parameters are associated with transformation of cells in vitro. However, with the exception of tumor formation in susceptible hosts, there is no other universal unequivocable proof of transformation if one wishes to be relevant to the in vivo situation. For example, nontransformed BHK21 hamster cells will grow in soft agar or in suspension when the culture medium is supplemented with pig serum rather than bovine serum. Thus, serum may influence the appearance of differences between normal and transformed cells. There are no quantitative systems that utilize epithelial cells. It has been considered important to transform epithelial cells because of the possibility that the action of the carcinogen on epithelial cells might differ from that of fibroblast cells. This point along with the realization that the majority of human cancers are of epithelial origin has been the impetus for a number of attempts to transform epithelial cells. Unfortunately, with rare exception, the tumors that result from treating so called epithelial cells in culture produce either fibrosarcomas or undifferentiated tumors. The significance of the results obtained with epithelial-like cells will become apparent when models become available which provide transformations that result in undisputable carcinomas with a relatively high efficiency. The problem is complicated by at least two factors. The first is concerned with the nature of epithelial cells and their differentiated state. The second factor concerns culture technique. The tissue culture conditions currently in use are ideal for fibroblast-like cells. Thus, although primary cultures may consist of or contain specific parenchyma or epithelial cells, they adopt wide biochemical differences not related to the tissue of origin because of the tissue culture conditions in which all genes necessary for differentiation are turned off. Two approaches which have been neglected for obtaining transformation are the use of organ culture and the host-mediated method. One problem with organ culture has been that the explants must be kept for periods of time that approximate the in vivo situation. The administering of carcinogens in vivo before transferring the target cells to tissue culture has resulted in bladder tumors, glia-derived tumors, and sarcomas when kidney material was used. The host-mediated system is currently being used with pregnant animals. Studies in Japan, Europe, and the United States, including this laboratory, are proving that a number of carcinogens which require metabolic activation are positive when this approach is used and negative when the chemicals are applied directly to the cells in culture. The frequency of transformation by chemical or viral carcinogens is controlled by a number of factors. Recent

studies show that chemicals, whether weak chemical carcinogens or noncarcinogens or physical agents, may modulate the transformation frequency that is observed. Although several mechanisms have been proposed for the initial events leading to neoplastic conversion of normal cells, the close association between the mutagenic and carcinogenic activities of a wide variety of chemical carcinogens has focused much of the attention on the interaction between chemical carcinogens and cellular DNA. The capacity of many chemical carcinogens to induce DNA damage that can be demonstrated by sedimentation in alkaline sucrose has prompted some investigators to consider the induction of DNA strand breaks as an assay method for chemical carcinogens. Our studies, based upon the incorporation of ^{3}H-TdR and subsequently assayed by autoradiography, indicate that no simple relationship exists between carcinogenic activity and induction of repair synthesis. Our review indicates that the critical parameter in screening for carcinogens may not be the measurement of the extent of repair induced by physical and chemical agents, but the remaining amount and type of unrepaired DNA damage.

Chromosomal abnormalities are a frequent concomitant of neoplasia, and although it is tempting to relate these mutations and alterations in chromatin (DNA) function to cancer, their relationship to the initiation or progression of carcinogenesis is unknown. Mammalian cells in culture, after interacting with chemical carcinogens, often exhibit chromosome damage consisting of breaks and exchanges of chromatid material. The pattern of damage of banded metaphases indicates that negative bands are especially vulnerable to the action of chemical carcinogens, probably because of differential chromatin condensation. Damage to individual chromosomes may be random or nonrandom, depending on the species. Cell death can be correlated with chromatid alterations that occur shortly after treatment with chemical carcinogens. There is also a correlation between mutagenic and carcinogenic activity of some chemical carcinogens and the frequency of sister chromatid exchanges. The question of whether specific chromosome changes are absolutely required for neoplastic transformation cannot be answered because of conflicting data and diverse results from studies even with known carcinogens. Cell transformation may occur without any visible chromosome changes. A universal specific numerical or visible structural chromosomal alteration is not necessarily associated with chemical or viral transformation. Chromosome changes are independent of the etiologic agents; different carcinogens may produce transformation associated with the same abnormal chromosomes, but not all transformed lines invariably exhibit the same abnormality, even with the same chemical. In some species, chromosomes having nucleolar organizer regions may be more frequently involved in numerical or structural deviations. Progressively growing tumors also may occur as a result of the proliferation of transformed cells without detectable chromosome changes, indicating that tumorigenicity need not be related to an imbalance of chromosome number or structure. Our studies indicate that chromosome changes are not essential for establishment of neoplasms but that karyotypic instability may result in response to selective growth pressures.

The absence of detectable viral expression by the parameters used is consistent with the interpretation that cells transformed by chemicals do not intrinsically express virus and that detectable virus expression is not necessary to

maintain their transformed state. Furthermore, no expression of hamster type-C viral RNA could be detected in cellular RNA from control hamster embryo primary fibroblasts, or from embryonic hamster kidney and liver. Thus, although it is possible that virus might be transiently expressed at some point prior to the acquisition of transformed phenotype, the weight of the data suggests that chemical transformation is neither accompanied by the continual expression of components of the hamster type-C virus, nor is such expression required for the maintenance of the transformed state. Consequently, the dose response relatinship of transformation of Syrian hamster cells by chemical carcinogen was investigated with and without pretreatment with X-irradiation. Different concentrations of chemical carcinogens were used with and without a constant amount of radiation. No transformation occurred with X-irradiation alone or the untreated controls, but X-irradiation has a transitory effect on transformation by chemical, with the maximum transformation frequency being observed when a 48 hr interval separates the X-irradiated insult and chemical carcinogen treatment. Since the addition of carcinogen less than 48 hrs to irradiated cells resulted in a lower degree of enhancement, X-irradiation cannot be considered an initiating agent in the usual sense. These studies indicate that transformation is due to a one-hit "phenomenon". Among the various experiments, the points deviate from the curve at different concentrations. A greater variation in transformation occurs among plates with larger doses of chemical carcinogen. Nevertheless, the two curves, with and without X-irradiation, can be described by one number since the ratio of the two ↗ is 8-9-fold. These results also indicate binominal variation which is a reflection of the number of cells at risk. Thus, the shapes of the curves demonstrate that transformation has a Poisson distribution and is consistent with a single hit rather than a large number of events.

X-irradiation and methyl methanesulfonate (MMS) are capable of enhancing transformation associated with a number of chemical carcinogens without altering cell cycle or the number of cell synthesizing DNA. Furthermore, the damage to DNA as identified by unscheduled DNA synthesis appears to be a transitory phenomenon since it is over within 6-24 hrs and yet the maximum enhancement occurs when a potent chemical carcinogen is added 48 hrs after MMS or X-irradiation. Therefore, the response in terms of unscheduled DNA synthesis was measured in cells which were treated at zero time with MMS and treated with N-acetoxy-2-fluorenylacetamide (AcAAF) at 24, 48, and 72 hrs later. This study was done using hamster embryo cells in arginine-free medium in order to stop DNA synthesis and cell replication. At the end of the respective incubation periods, the dishes were rinsed and treated with ^{3}H-thymidine in arginine-deficient medium. After development, the average grains/nucleus was determined. The results of the combination treatment were compared to that obtained as a result of repair from MMS alone and AcAAF treatment alone. The comparison of the results obtained indicate that at 24 hrs after MMS, the two treatments result in repair which is less than that obtained by the individual treatments. At 48 hrs, the two-treatment combination is equal to that obtained by either treatment. At 72 hrs, results obtained with sequential treatments are much greater than those from either treatment alone. In this last instance, the results may be considered to be synergistic. The current working thesis is that the enhancement and transformation obtained at 48 hrs

is the result of the DNA damage which is observed at 72 hrs in inhibited cultures. Most likely, the difference between results at 72 hrs in terms of repair and transformation at 48 hrs is due to the difference in the metabolic state of the cells, since unsynchronized cells were used for the transformation experiments.

The identification of sister chromatid exchanges (SCE) may be a means for identifying environmental carcinogens. New, simple, reliable and highly sensitive methods, based on 5-bromodeozyuridine (BrdU) substitution in DNA visualized after fluorochrome or Giemsa staining, were used on the V79-4 cell line of male Chinese hamster lung fibroblasts. Four classes of chemicals were used: (1) Those which are carcinogenic per se or metabolizeable by V79 cells to a mutagenic and presumably carcinogenic form: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), AcAAF, N-hydroxyfluorenylacetamide (hydroxy-FAA), and 4-nitroquinoline-1-oxide (4NQO); (2) Chemicals which are active carcinogens in vivo but require metabolic activation by enzymes absent from V79-4 cells: urethane, N-2-fluorenylacetamide (FAA), Benzo(a)pyrene (BP), 7,12-dimethylbenz(a)anthracene (DMBA), and 3-methylcholanthrene (MCA); (3) Chemicals which are mutagenic in micro-organisms but are putatively weak or not at all carcinogenic in vivo: proflavine and acridine orange; (4) Nonmutagenic noncarcinogenic chemicals: hydroxyurea, methylene blue, pyrene, perylene, and phenanthrene. The chemicals in class (1) caused a significant increase in SCE frequency; 4NQO and hydroxy-FAA produced a positive dose response effect. The intercalating dyes, class (3), proflavine and acridine orange, increased SCE and aberration frequencies. The inability of the nonmutagens and noncarcinogens (class 4) to induce SCE is perhaps not surprising since all the agents which are known to induce SCE also cause damage to DNA and other cell constituents while those in class (4) do not. Precarcinogens (class 2) do not damage DNA in the absence of metabolic activation. Three carcinogenic (BP, DMBA, and MCA) and two noncarcinogenic (pyrene and phenanthrene) aromatic polycyclic hydrocarbons were retested for ability to induce SCE and aberrations in V79 cells when grown on an irradiated feeder layer of secondary Syrian hamster embryonic cells. The feeder layer did not affect the SCE level in the control sample, but reduced the incidence of aberrations. In the presence of the feeder layers, the BP and MCA-treated cells exhibited a significant increase in SCE frequency but no increase in aberration frequency. DMBA (1.0 µg/ml) delayed cell cycle progression; the majority of metaphases observed 24 hrs post-treatment were still in M_1, 70% of these metaphases having damage. In the rare M_2 metaphases seen, the frequency of SCE observed was similar to that obtained with BP and MCA. Neither of the noncarcinogenic polycyclic hydrocarbons increased the SCE frequency per chromosome. The relationship between SCE and chromosomal aberrations remains unclear. Of the chemicals tested, only those which react with DNA, either directly or after metabolization, induce SCE. Most such chemicals are carcinogens. These results lead to the conclusion that SCE tests would be useful in a screening program for genetically active chemicals. However, since SCE are induced by proflavine and acridine orange, for which no strong evidence of carcinogenicity exists, the testing of a much larger number of carcinogens and noncarcinogens is necessary before any conclusions can be drawn as to the predictive value of SCE analysis for the identification of carcinogens.

The induction of stable, structural, or numerical chromosome alterations has been demonstrated in Syrian hamster cells transformed by Simian adeno virus, SA7, or by sequential treatment with chemical carcinogen and SA7 virus. The abnormal chromosomes or numerical deviations from chromosome pairs identified by banding methods were considered to be random. The fate of the virus genome in SA7 and chemical carcinogen and SA7 Syrian hamster cells was investigated using the in situ molecular hybridization method. This procedure allows the detection of viral nucleic acid on fixed preparations. Fixed nuclei and metaphases obtained from 20 clones or reclones transformed with SA7 or from SA7 virus plus chemicals were hybridized with cRNA and examined after autoradiography. Viral nucleic acid was detected in 50% of the samples examined. The label was randomly distributed over nucleus or chromosomes. A decrease of SA7-DNA occurred with subculturing. This may represent a loss of some information from the entire population or a selection of cells containing little or no SA7-DNA during subculturing. The second explanation seems to be more likely because the cells that were recloned at least twice had no viral nucleic acid as indicated by in situ hybridization method, despite the fact that an average of one genome per cell was found with a biochemical procedure. Eight different clones were examined for SCE frequency to determine whether or not viral presence affects the stability of chromatid material. No significant differences in SCE frequency was found between clones transformed with SA7 alone versus SA7 plus DMBA, between the above cells whether repeatedly subcultured and early passages and, finally, between negative and positive SA7-DNA clones. No correlation between the presence of SA7-DNA and tumorigenicity could be established since all transformed clones produced tumors when injected in appropriate hosts.

Significance to Biomedical Research and the Program of the Institute: The prevention of cancer in humans depends to a large extent on removing potentially harmful environmental agents. Accepted testing in animals is an insurmountable task. The proper evaluation of a chemical to determine if a chemical is carcinogenic requires a large number of animals and over two years, not including the time required to analyze the accumulated data. Bioassays capable of screening potential carcinogens in a relatively short period of time at a fraction of the current costs are essential to a workable testing program. The need to study chemical carcinogenesis in vitro is logical when one realizes that carcinogenesis is really a problem in cell ecology. Consequently, in vitro carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of in vivo problems. Therefore, in vitro carcinogenesis has wide applications in terms of studying compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course of Project: The conditions which are responsible for or can alter in vitro transformation with specific emphasis on the requirements of human fetal cells will be studied. UV, X-rays, and combinations of carcinogens of different classes, the combination of in vivo - in vitro systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity

and transformation will be considered. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04673-06 BGY

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogens In Vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Evans	Head, Tumor Biology Section	BGY NCI
OTHER:	J. A. DiPaolo	Chief, Biology Branch	BGY NCI
	J. O. Rundell	Staff Fellow	BGY NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biology Branch, Carcinogenesis Program

SECTION
Tumor Biology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Carcinogens from diverse chemical classes induce neoplastic transformation of guinea pig fetal cells in culture. The transition to the neoplastic state often occurs in a stepwise manner. Morphological transformation frequently precedes neoplastic transformation, the ability of the cells to produce progressively growing tumors in syngeneic guinea pigs, by several months or more. Neoplastic transformation, furthermore, occurs four or more months after carcinogen treatment. Alterations in plating efficiency, growth rate, and chromosome number and structure are not necessary for the development of neoplastic transformation. The ability of transformed cells to grow as colonies in agar, susceptibility of the cells to cytotoxic effects of peritoneal exudate cells from nonimmune guinea pigs, intradermal skin reactivity of transformed cells in nonimmune guinea pigs, and secretion of large amounts of plasminogen activator are characteristics that correlate well with neoplastic transformation. These cell properties are useful indicators of the tumorigenic potential of guinea pig cells transformed by chemical carcinogens and for study of somatic cell alterations essential for the development of the neoplastic state.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The primary objective of this project is to establish conditions and methods for the quantitative study of in vitro chemical transformation of guinea pig cells. The investigation complements the previously developed and current in vitro chemical carcinogenesis system established within the Biology Branch employing Syrian hamster embryo cells and the tumor immunology program utilizing in vivo chemical carcinogen induced tumors developed in strain-2 guinea pigs.

The specific objectives of the project are two-fold:

1. The development of a rapid assay system for chemical carcinogens suitable for the screening of compounds and populations. As an inbred strain with a low incidence of spontaneous malignancy, strain-2 guinea pigs offer a mammalian system in addition to the Syrian hamster for the analysis of chemicals as potential carcinogens and investigation of the mechanisms of chemical carcinogenesis at the cellular level.

2. The development of assays to determine whether in vitro chemical transformation is accompanied by the appearance of tumor specific antigens and/or other alterations associated with the neoplastic state. The Biology Branch has extensive experience with tumor immunology in strain-2 guinea pigs. A variety of in vivo and in vitro immunological techniques are presently being utilized. The availability of these techniques and the syngeneic system offered by the inbred strain-2 guinea pig make this system a better choice for immunological study at this time than the Syrian hamster. The development of neoplastic cells transformed in vitro by chemical carcinogens will permit assessment of cell surface antigen alterations associated with chemical carcinogens as well as further understanding of in vivo tumor immunity in strain-2 guinea pigs as a model system for carcinogenesis and tumor immunology in general.

Methods Employed: The quantitative in vitro chemical carcinogenesis system developed employing Syrian hamster embryo cells within the Biology Branch has been utilized throughout this study. Cells are freshly isolated from whole or specific organs of strain-2 guinea pig embryos or fetuses and are grown in the presence or absence of irradiated rat, hamster or strain-2 guinea pig feeder cell layers. The cells as a mass culture or as individual cells are exposed to chemical carcinogen subsequent to seeding the cells in the Petri dish.

Major Findings: 1. Carcinogens from diverse chemical classes including the polycyclic aromatic hydrocarbons benzo(a)pyrene, 3-methylcholanthrene, and 7,12-dimethylbenz(a)anthracene, the amide N-acetoxy-N-2-fluorenylacetamide, the nitroso compound N-methyl-N'-nitro-N-nitrosoguanidine, the nitrosamine diethylnitrosamine and aflatoxin B_1 induce neoplastic transformation of strain-2/N fibroblast-like guinea pig cells in culture. Carcinogenesis can be initiated by application of the carcinogen directly to cells in

culture except for compounds such as diethylnitrosamine that require metabolic activation. These compounds and the direct acting carcinogens are effective when the cells are exposed in vitro with the host mediated in vivo - in vitro method.

2. Carcinogenesis utilizing guinea pig cells proceeds in discrete stages that frequently are displayed for extended periods of time. Morphological alterations relative to controls develop shortly after carcinogen exposure and persist for months. After four or more months morphologically altered cells proceed to the stage of morphological transformation characterized by random growth and piling up of cells. Neoplastic transformation, the ability of cells to produce tumors when inoculated into host animals, may be demonstrated at the time of morphological transformation or frequently may require additional months of subculture. This stepwise progression of the carcinogenic process occurs following treatment of cells obtained from fetuses in midterm and near-term gestation and with cells isolated from neonatal guinea pigs.

3. A number of somatic cell alterations including the ability to form colonies in agar, secretion of plasminogen activator, intradermal skin reactivity in nonimmune syngeneic animals and susceptibility to the cytotoxic activity of lymphotoxin develop in close proximity or concomitantly with neoplastic transformation. These cell properties provide useful quantitative means to identify neoplastic transformation of cells in culture and to investigate cellular characteristics essential to the development of the neoplastic state.

4. The neoplastic state is associated with a quantitatively specific susceptibility to lymphotoxin. There is a wide range of lymphotoxin susceptibility among different neoplastically transformed cells that is independent of the origin of the cells or the initiating chemical carcinogen. Neither chromosomal alterations nor detectable tumor specific cell surface neoantigens are necessary for neoplastic transformation; furthermore, these factors are unrelated to lymphotoxin susceptibility.

Significance to Biomedical Research and the Program of the Institute: This project provides an unique in vitro model system with several distinguishing characteristics for the study of chemical carcinogenesis. In vitro chemical neoplastic transformation of strain-2/N guinea pig cells will provide another assay system in addition to the Syrian hamster and established mammalian cell lines for screening of chemicals as potential carcinogens and broaden our understanding of carcinogenesis at the cellular level through inspection of intracellular and cell surface alterations accompanying neoplastic transformation. Development of this new model offers an additional avenue for studying the process of carcinogenesis using a species with established tumor biology in which spontaneous transformation has not been seen, which possesses well defined immunological parameters, and in which discrete stages in the transition from the growth controlled to the neoplastic state may be studied.

Proposed Course of Project: Conditions and methods will continue to be refined toward attaining optimal development of in vitro transformation of strain-2 guinea pig cells as a reproducible, quantitative and reliable assay for

potential chemical carcinogens. Investigations will continue in defining the events, factors and cellular alterations accompanying the stages, i.e. morphologic alteration, morphologic transformation and potential for neoplastic growth, in the transition from the growth controlled to the neoplastic state. Of immediate interest are the properties of cells with neoplastic potential that permit them to grow as colonies in agar and to be susceptible to the cytotoxic and growth inhibitory activities mediated by nonimmune leukocytes in the absence of or independent of the presence of detectable tumor specific cell surface antigens. The elucidation of these relationships will provide further understanding of the mechanism of chemical carcinogenesis at the cellular levels of tumor establishment and immunity in the host animal.

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SUMMARY REPORT

CARCINOGEN BIOASSAY AND PROGRAM RESOURCES BRANCH

July 1, 1976 through September 30, 1977

"Plans, develops and implements a coordinated research program to (1) test chemical and physical agents for carcinogenicity by standard bioassay methods; (2) establish the necessary methods and logistics for the bioassay program; (3) develop and provide animal, chemical and information resources and data analysis to the Carcinogenesis Program; (4) monitor animal care and welfare in the intramural and collaborative contract programs; (5) develop and standardize selected animal models for carcinogenesis research; and (6) conduct studies aimed at determining the cocarcinogenicity of radiation with chemical agents."

The Branch, established in April 1973, expanded in 1975 with the addition of Dr. Kenneth Chu, Chemist and Ms. Joan W. Chase, Technical Information Specialist. In 1976, Dr. J. Fielding Douglas, Biochemist was added to the staff as Expert. In 1977, the Testing Program received 31 positions, of these 15 were assigned to the Branch. Additional positions will be available for next year. Active recruitment is underway to fill all vacant positions.

The Branch provides support for the entire Carcinogenesis Program, and also has major responsibilities in two areas of the collaborative program: the Bioassay Operations Unit and the Information and Resources Program. The Branch provides scientific expertise and leadership for the development of a carcinogenesis bioassay program, which has now become a well recognized national resource.

While the Branch is not formally divided into sections, its activities are primarily related to three main subjects: carcinogen bioassay, information activities, and animal resources and care. Reports on these areas are as follows:

(1) Carcinogen Bioassay - This year has seen the accomplishment of a major commitment to this program area: the development of a reporting system on over 200 chemicals which have been tested in the bioassay program. This system involves the collating of data and information, report writing, evaluation of the report, its publication and dissemination. The system has involved the creation of an NCI "Data Evaluation Group" (DEG) to evaluate all Technical Reports and recommend revisions as needed.

General aspects of the Branch's activities relate to the development of improved methodology and improved predicitability of carcinogen bioassay in terms of various species, including man.

Specific aspects of the Branch's activities relate to the (a) identification and selection of chemicals for bioassay; (b) acquisition of information on use and characterization of the agent being tested; (c) establishment of logistical capabilities for testing; (d) acquisition, characterization, and purification of these agents; (e) establish testing protocols through the NCI Experimental Design Group; (f) implementation of the appropriate bioassay tests; (g) monitoring test progress and performance; (h) development of a data bank to include results of bioassay testing; (i) analysis and evaluation of the test results; (j) report writing; and (k) evaluation of reports through the NCI Data Evaluation Group; and (l) decision on further action required concerning the tested agents. The bioassays are conducted through contracts within the Bioassay Operations Unit. Members of the Branch provide direction and management to those studies as well as expertise and support in the development of the necessary resources for animals, chemicals and information systems. The main categories of compounds on test are pesticides and related chemicals, drugs and cosmetics, chemical intermediates and reagents, dyes and related chemicals, solvents, food or food additives, and rubber and paint chemicals.

Procedures for the selection of chemicals for carcinogenesis bioassay were modified during the year in collaboration with other Program staff and with the Prime Contractor. Emphasis is now placed on review of classes of chemicals rather than individual compounds. Information resources developed by the Branch in conjunction with the Information and Resources Unit have provided the required basis for decisions on testing.

The Experimental Design Group (EDG) was re-organized in November, 1976 assigning NCI members the responsibility for establishing objectives, giving guidance and making decisions. The prime contractor members were assigned responsibility for operations, support and advice. This is consistent with the concept that experimental design of carcinogen bioassay is a primary responsibility of NCI.

The EDG presents its findings to a subcommittee of the newly created National Clearinghouse on Environmental Carcinogens for technical advice. However, the EDG is administratively and technically responsible directly to the Chief of the Branch.

The Group is charged with the following responsibilities:

(1) Establish basic objectives of the test and data elements required for each chemical considered.

(2) Establish specific experimental design to obtain the required data and provide a written outline of the protocol recommended.

(3) Maintain close interaction with Data Evaluation Group; identify general objectives and problem areas in experimental design and formulate recommendations.

The Data Evaluation Group (DEG) was established in August, 1976 to meet the objectives of efficiently reporting results of carcinogenesis bioassays while maintaining a rigorous and systematic evaluation of the experimental data. The DEG presents its technical findings to a subcommittee of the newly established National Clearinghouse on Environmental Carcinogens. However, the DEG is administratively and technically directly responsible to the Chief of the Branch.

The Group is charged with the following responsibilities:

(1) Establish basic data requirements for specific type of reports.

(2) Establish criteria for data review and evaluation.

(3) Review and evaluate collated data as to its biological and statistical significance in the assessment of the possible carcinogenicity of a specific chemical to experimental animals.

(4) Formulate recommendations as to data needs, limitation of interpretation and nature of report to be written.

Maximum tolerated doses (MTD) for chemicals going on test are estimated through review and evaluation of subchronic data by toxicologists from the Branch, prime contractor and subcontractors. The objective is to set MTDs that result in good survival of treated experimental animals without the need to change doses during the progress of the chronic treatment.

The Carcinogenesis Bioassay Data System (CBDS) is used to collect, monitor, store, and report experimental data and results from bioassay operations. This year, special attention has been given to generating reports and analyzing data in order to accelerate reporting of carcinogenesis bioassay results. This involves producing automated sections of preliminary and final reports on specific chemicals. It includes a description of the experimental design, tumor and non-tumor incidence, summary tables of lesions by anatomic site, individual animal pathology, table of tissues examined, survival curve analysis and growth curve analysis.

Automated statistical analysis program of growth and survival curves, and specific tumor types was established using unadjusted and time-adjusted techniques. The unadjusted methods include the Armitage and Cox test for linear trend and Fisher-Irwin Exact test while the time-adjusted methods involve the Cox-Tarone test for trend. Many of the data tables, growth and survival curves, and data analysis are included directly in the technical reports now being generated at an accelerated rate.

One of the most pressing concerns in the bioassay program is the need to fully document and make available the details of the methodology, test performance and results of each chemical bioassay. While usual reports

in the literature or at conferences will still be encouraged, the NCI Carcinogenesis Technical Report Series has been established to provide the necessary medium for all public disclosure of bioassay detailed reports.

Contracts with Tracor Jitco and Mitre Corporation provide most of the effort in collating data and preparing the technical reports. Branch staff personnel assure input of data from the Carcinogenesis Bioassay Data System (CBDS). The critical evaluation and review of technical reports now represents a major undertaking of the Branch. Procedures have been developed for the release of technical reports in such a way that all interested parties, Government and private, receive the results systematically. The Office of Cancer Communications announces the availability of the reports in a press release, along with an announcement in the Federal Register. These procedures were approved by NCI and DHEW.

This year information requests have greatly increased partly because of increased knowledge of the chemicals on test and partly in response to the Freedom of Information Act as amended in 1974. The use of the Freedom of Information Act to gain access to pathology slides, computer data tables, progress reports, and draft reports has created extensive work demands. The release of preliminary or partial results of the bioassay prior to their scientific review and analysis is a potential source of misunderstanding and must be handled with proper warnings of the limits of interpretation of such data.

(2) Program Information Activities - Data and information activities are oriented to the identification, gathering, collating, analyzing and reporting of material relevant to the Bioassay Testing Program. Branch staff contribute considerable effort to the production of the "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" (PHS-149), and Carcinogenesis Abstracts. Review and monitoring of the content and also use and maintenance of the mailing keys of both of these activities were conducted by branch personnel. Contractual support was given to the International Agency for Research on Cancer (IARC) to produce the IARC Monographs on "The Evaluation of Carcinogenic Risk of Chemicals to Man" and to Stanford Research Institute to collect and analyze data and information on chemicals that impact on man and the environment. Searches for data and information were performed for the Carcinogenesis Testing Program staff using data bases maintained by the National Library of Medicine (Toxline, Medline), Smithsonian Science Information Exchange, National Technical Information Service, Environmental Mutagen Information Center, Environmental Teratology Information Center. Staff has been formally collaborating with the Office of the Director in the development of the International Cancer Research Data Bank and the Carcinogenesis Cancer Information Dissemination and Analysis Center.

(3) Animal Resources and Care - Specific aspects of this activity relate to the intramural and contract-supported programs for (a) procurement of animals; (b) assuring compliance with animal welfare laws and the

DHEW Guide for Care and Use of Laboratory Animals; (c) assistance to the Carcinogenesis staff with animal health and husbandry problems and plans for animal facility modifications; (d) contributions to the development of animal models for cancer research as well as to the collection and dissemination of baseline information on laboratory animals, and (e) identification of animal variables in the in vivo program.

Animal Resources personnel assist in planning animal needs as far in advance as possible, coordinating these needs with animal suppliers and providing information on the characteristics and availability of laboratory animals. It also assists and advises on the compliance with the Animal Care and Welfare standards for both contract and intramural programs.

(4) Other Activities - The need to correlate individual bioassay results and gain insight into the mechanisms of action has led the Branch to explore methods for correlating structure to carcinogenic activity. In particular, the Branch is examining methods, such as regression analysis, pattern recognition, and discriminant analysis. In addition, the Branch is looking at methods to incorporate metabolic considerations in the structure-activity correlation.

Development of methodology for the determination of the metabolic spectrum of endogenous materials in plasma of rats is being conducted. This will serve as a basis to evaluate the extensive concern that maximum tolerated doses should not overload the metabolic processes as reflected by changes in metabolism of both the test compound and endogenous materials in plasma.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04539-05-CBPR

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Information and Data Activities of the Carcinogenesis Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Siegel, Research Biologist	CBPR, NCI
Others:	J. Chase, Technical Information Specialist	CBPR, NCI
	K. Chu, Chemist	CBPR, NCI
	C. Cueto, Acting Chief	CBPR, NCI
	M. Linhart, Programmer/Analyst	CBPR, NCI
	R. Griesemer, Acting Head Bioassay Testing Program	CG, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Carcinogen Bioassay and Program Resources Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Identifies, designs, implements and maintains data and information activities relevant to the scientific and scientific/administrative concerns of the multi-discipline science Carcinogenesis. In so doing seeks to increase standardization of the approaches used to identify, gather, collate, store, retrieve, evaluate, and report data and information as well as to insure an effective and efficient linkage of such related internal activities with those external to the program. Emphasis is also placed on interpreting the relationship of the physics of a molecule and effects which are induced in biological systems, and also on predicting the possible metabolic pathways of exogenous chemicals in these systems. Special interest is directed towards information on the production, use and distribution of chemicals as related to possible environmental and human hazards.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To provide data and information through computer or non-computer systems that can serve the needs of scientists and scientist/administrators involved in Carcinogenesis and Carcinogenesis Bioassay Testing research activities. To this end it has been necessary to analyze the needs of the program or program related projects to determine the best cost effective method of responding to essential data and information needs. The needs range from identifying relevant material to its gathering, collating, storing, retrieval, analyzing and reporting in useful format(s). By establishing and monitoring data and information activities internally and also identifying relevant activities external to the program, the information needs of staff members relevant to their own responbilities can be met in an economical manner.

Methods Employed: While branch personnel perform most of the tasks associated with information and data acquisition activities, the requirements exceed in-house capabilities. Contractors are used to interface with branch members to augment information needs of specifically defined projects. Activities which can be described as data and informational in content and scope consist of the following components: identification, gathering, collating, storing, retrieving, abstracting and reporting. Projects in this area of the program can consist of one or a combination of these components. The journal Carcinogenesis Abstracts consists of all of these components, except analysis, while the IARC monographs include evaluative and interpretive components. Coordination of operational, experimental, and projected designs of informational activities is required for efficient, effective and economical responses to the program's data and informational requirements. Emphasis has been placed on methodology which explores the relationship of the physics of a molecule to the effects it may produce in biologic systems. An important dimension to this work has been a project using computer technology to outline probable metabolic pathways of exogenous chemicals in biologic systems. These studies are being integrated with projects that determine and analyze the production, distribution and use pattern of chemicals significantly impacting on humans and the environment.

Major Findings: A significant increase in acquired program relevant data and information has been made as documented by the levels of activity associated with Carcinogenesis Abstracts, The Environmental Mutagen Information Center, The Environmental Teratology Information Center, the PHS-149 Publication Series "Survey of Compounds Which Have Been Tested for Carcinogenic Activity", and the International Agency for Research on Cancer Monographs entitled "Evaluation of the Carcinogenic Risk of Chemicals to Man."

Significance to Biomedical Research and the Program of the Institute:

The multi-discipline composition of research associated with the sciences of carcinogenesis and bioassay testing technology makes it mandatory that

both proven and more advanced methodologies of informational science be applied to increase the amount and rate of intellectual understanding and articulation among the scientists and scientist/administrators from the various disciplines.

Increasing the amount of understanding and articulation of all of the Program's activities increases the ability of scientists and scientist/administrators to make value judgements and decisions concerning the direction of specific projects and programs. Decision making thus has been enhanced by data and information activities which themselves are continually critiqued as to usefulness.

Proposed Course of the Project: The project will continue to generate reports, publications or special products. These are evaluated as to content, format, and usefulness to the federal, academic and industrial communities concerned with scientific and regulatory aspects of potential carcinogens.

The Program will continue to increase its capability to link the various activities so as to insure that when a data and information problem is addressed, the answer will be reflective of an analysis performed on a set of facts efficiently and comprehensibly identified, gathered and collated.

In regard to program management, development will continue on the computer based system (MIS) to aid staff members in controlling both contract and intramural activities. This system continues to have the support and encouragement of the Director's Office at both the NCI and Division levels. Coordination with other government and non-government research and research/administrative activities, continues to increase as a result of cooperative efforts. This is exemplified by the following cases:

(1) Environmental Protection Agency

Cooperative efforts on -

Information File Standardization

Technology associated with Chemical Selection and Ordering for Priority

(2) The HEW Committee to Coordinate Toxicology Research
Subcomittee to Coordinate Toxicology Information Project

Cooperative efforts on -

Laboratory Animal Data Bank

Tox-Tips

Chemical Information File Coordination

Product Classification Codes

"The Information and Data Activities of the Carcinogenesis Program" as a basic project is sensitive to the requirement that data and informational activities be an integral part of program and data management systems. These activities, properly integrated, allow a continual monitoring and evaluation of studies being conducted by the program. The project's management capabilities would allow program scientists and scientist/administrators to maintain a close review of their intra and extramural activities and facilitate better direction and control of the studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04541-05-CBPR

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Animal Resources

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. P. Cameron, Veterinary Officer, Director Grade	DCCP, NCI
	Clarence Reeder, Animal Husbandman	DR&D, NCI

COOPERATING UNITS (if any)
Frederick Cancer Research Center; Genetics Unit of the Small Animal Section, VRB, DRS: Mammalian Genetics and Animal Production Section, DCT

LAB/BRANCH
Carcinogen Bioassay and Program Resources Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.3	1.5	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program resource continues to supply sufficient numbers of animals of satisfactory health status to support the bioassay needs. In addition, effort is expended to develop new animal models and/or documentation that will enhance their use in the intramural and extramural program areas. Species and strains of animals that are not otherwise available in adequate supply are provided through a variety of mechanisms, i.e., Frederick Cancer Research Center and contracts of the Division of Cancer Treatment.

The husbandry aspects of the bioassay extramural contracts have received a great deal of attention and have been significantly upgraded. In conjunction with the appropriate prime contractor staff members, variables impacting upon the proper execution of the protocols are identified and methodically eliminated in a manner that will not disrupt the ongoing program.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) Plan and affect Procurement of animals used in the Carcinogenesis intramural and contract programs; (2) Assure compliance with the Animal Welfare Laws and the DHEW Guide for the Care and Use of Laboratory Animals; (3) Plan and support the development of animal models for cancer research as well as to collect and provide information on laboratory animals; (4) Provide general guidance on facility construction/ renovations, and methodology to contractors; and (5) Utilizing the available contract capabilities, identify and seek to eliminate variables contained in the In Vivo system.

Methods Employed: The number of animals required for the various aspects of the Carcinogenesis Program are estimated as far in advance as possible by the program staff, staff of the Bioassay Program Prime Contractor, and the staffs of the various contracts.

The bulk of these needs are met by the Animal Farm at FCRC; the nucleus breeding stock for that effort continues to be supplied by the Genetics Unit of the Small Animal Section, VRB, DRS. A modest number of animals of varying species, strains, and stocks continue to be supplied by the Mammalian Genetics and Animal Production Section, DCT, under the mechanism of fund transferral from the Carcinogenesis Program to DCT. It is anticipated that this year will be the last in which the mechanism of fund transferral will be required. In subsequent years, animals from the two commercial production contracts now beginning to supply the bioassay program (Charles River Breeding Laboratory and Harlan Industries) will be utilized by the DCT Program in equal numbers to the demands made by the Carcinogenesis Program upon the DCT contracts and the prior funding activity will be eliminated.

The needs of the Prime Contractor for the Bioassay Program, Tracor Jitco, other contractors and the intramural staff are transmitted to the Mammalian Genetics and Animal Production Section, DCT. Members of that unit, in addition to assuming the responsibility for acting as Project Officers for the animal production contracts of the Carcinogenesis Program, coordinate the distribution of animals from the contract, FCRC animal farm, and the MG&APS contracts if need be to the using laboratory.

The contract with the ILAR, NRC/NAS has resulted in a publication, "Standards for Successful Chronic Rodent Studies". This publication has been widely disseminated by the ILAR office. We have been informed that it is part of the syllabus for the FDA's course in training of animal laboratory inspectors.

The contract with Dr. Ulrich Mohr, Medizinische Hochschule, Hannover, West Germany, to develop the European Hamster as a cancer model has been redirected by other elements of the Carcinogenesis Program. The original purpose to establish that animal as a laboratory animal is considered completed and it is anticipated that a contract will be awarded to develop

it as a model for pancreatic cancer. Dr. Mohr and his co-workers have written an anatomical atlas for this hamster and this program has subsidized its development into a project that has been delivered to the Government Printing Office for publication as an aid in the utilization of this animal model.

Major Findings: The major effort of the resource area has been to insure the availability of sufficient animals of extremely high health status to adequately support the Bioassay Program from the FCRC Animal Farm. In most instances, the production levels reached by that unit have sufficed for the Bioassay Program and the FCRC Bioassay Program demands. The Charles River and Harlan Industries contracts have now developed to the extent that a good share of the Fischer 344 requirements can be met by those companies by the end of FY 77 and the mouse demands by mid FY 78.

Due to the lack of suitable and available animal breeding space and available staff time, the European Hamster Colony has been dispersed. The animals have been allocated to one contractor (Eppley Institute, Omaha, Nebraska) which has had a great deal of experience with Syrian Hamsters and to the two commercial organizations which expressed an interest in acquiring nucleus breeders. It is anticipated that a few European Hamsters could be commercially available sometime in FY 78.

Significance to Biomedical Research and the Program of the Institute:

Biomedical research, to a very large degree, depends upon the selection of the proper animal model, the supply of sufficient numbers of healthy animals to satisfy the protocols and the correct care of the animals while under test. These factors are especially important to the extensive Bioassay Program in which animals are held for the greater part or all of their lifespan to study the carcinogenic influences, midway or later in this type of study, represent a significant loss in time and money. It is essential that the staff supply the proper quality and number of animals to the program and monitor their care both in the intramural and extramural programs.

Proposed Course of the Project: The project will continue as defined. The extramural, and to a more limited extent, the intramural programs will be serviced in the areas of animal supply, advice as to husbandry practices and the development of needed animal models in collaborative efforts.

A significant amount of time was expended monitoring the development of the laboratory animal data bank under the general direction of the National Library of Medicine. A computer program has been developed and emphasis will be directed to the accumulation and processing of required data elements.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04564-03-CBPR

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogen Bioassay Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Cueto, Acting Chief	CBPR NCI
Other:	T. Orme, Manager, Bioassay Operations Unit	CBPR NCI
	J. F. Douglas, Expert, PI for Prime Contractor	CBPR NCI

COOPERATING UNITS (if any)
Field Studies and Statistics Program; Office of the Scientific Coordinator for Environmental Cancer, DCCP

LAB/BRANCH
Carcinogen Bioassay and Program Resources Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and evaluate carcinogenic hazards of chemical agents in the environment and to conduct research to improve methodology of carcinogenesis bioassay and increase its capabilities as a predictive science.

For the most part the carcinogenesis tests consist of using standardized long-term animal bioassays in which both sexes of two species are exposed to the agent for a major portion of its lifespan, i.e., 18-24 months. Usually two dose levels are used with extensive pathology examination performed on all animals. The majority of the actual testing is conducted through contracts with the Program staff responsible for developing the required resources, protocols, monitoring of progress and establishing methods of reporting results.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The primary objectives of the program are to identify, using long-term animal bioassays, the potential carcinogenic properties of chemicals to which humans may be exposed and to plan and conduct research to improve techniques used in carcinogenesis testing so as to increase the bioassay's capability as a predictive science.

Methods Employed: The majority of the studies are conducted using long-term animal experiments in which both sexes of two species, usually rat, mouse or hamster, are exposed to the test material for a major portion of the animal's lifespan, usually 18-24 months. Following sacrifice the animals are carefully necropsied and approximately 30 tissues of each animal are subjected to microscopic examination. Routinely the material is administered by the route of exposure commonly encountered by man with two dose levels used, the high being the maximum tolerated dose (MTD). Considerable planning is required to coordinate implementation of the study; this involves procurement of animals, chemicals, specific information needed on formulation of chemicals and laboratory resources required for an efficient operation. As resources and funds are not available to test all chemicals introduced into the environment, a judicious selection must be made. The Chemical Selection Working Group of NCI selects chemicals for testing mainly on the basis of human exposure, production data, chemical structure and the lack of carcinogenic bioassay testing data. Detailed dossiers for each chemical considered are prepared. Nominations of chemicals come from various sources including other Federal agencies. Once selected and approved for testing, the chemicals must be procured and analyzed. The analyses of the chemicals and their formulations is performed by contract with commercial laboratories or special research centers having the required facilities and scientific staff.

In addition to providing for test facilities and chemical resources, animal needs must be forecast well in advance. The primary animals used in the Bioassay Program are the Fischer/344 rats and the B6C3F1 hybrid mouse (C57B1/6 x C3H). Most of the animals have been supplied by the Frederick Cancer Research Center. Commercial contracts have also been established for this purpose. Animals on long-term tests must be maintained under strict environmental surveillance to prevent the introduction and spread of infectious diseases that can quickly decimate rodent colonies or possibly modulate experimental findings. The chronic testing must be carefully planned to assure that the proper experimental design is used and that the bioassay is conducted under optimal conditions. The Experimental Design Group of NCI has the primary responsibility of establishing objectives, giving guidance and making decisions in terms of protocol and experimental design. Further details of this approach are given under the Branch Summary Report. Monitoring of the quality and progress of individual studies are through visits and reviews, the Carcinogenesis Bioassay Data System (CBDS) and routine progress reports.

It is important that bioassay data be recorded, analyzed and reported properly. Data is submitted, stored and retrieved by use of the CBDS.

Contractors are encouraged to submit the results of their testing to scientific journals having wide dissemination to the scientific community. As journals often will not accept detailed carcinogenesis bioassay data due to the bulk of the results, a Technical Report Series has been initiated. It is important that the results of these tests be properly tabulated and analyzed prior to release to the public or other agencies. For this reason, all bioassay data must be reviewed, approved and released through the Program.

The Pathology Working Group, although not structually within the Branch, has the responsibility of verification of pathology diagnosis and tabulations of lesions as well as verification and acceptance of the pathology narrative describing and evaluating the lesions.

The Data Evaluation Group of NCI was established in August 1976 to meet the objectives of efficiently reporting results of the carcinogenesis bioassay through the Technical Report Series while maintaining a rigorous and systematic evaluation of the experimental data. Further details on this subject are presented in the Branch Summary Report.

Procedures have been developed to provide for the release of Technical Reports in such a way that all interested parties, government and private, receive the results systematically. The Office of Cancer Communications distributes the reports and announces their availability in a press release, along with an announcement in the Federal Register. These procedures were approved by NCI and DHEW.

Major Findings: Extensive emphasis was placed this year on reporting the results of the bioassay obtained during the past three years. Draft reports of approximately 40 chemicals were presented to the DEG for evaluation and of these ten have been published and disseminated through the Office of Cancer Communications. The balance of the reports (30)should be ready for publication and distribution within the following three months. The reports present positive carcinogenic findings for some compounds and negative findings for others as determined within the limits of a successful experiment using standard carcinogenesis bioassay protocol. A few of the reports present questionable results due to experimental failure or marginal data.

Significance to Biomedical Research and the Program of the Institute:

Protecting the public from chemical carcinogenic hazards has been clearly recognized as an urgent and essential task in our society and a high national priority for this mission has been indicated by the National Cancer Act,

Congress and the Administration. One of the major program activities is the identification of carcinogenic activity of selected agents by bioassay testing. The results of this testing program are utilized in the establishment of public policy by regulatory agencies in regard to utilization of test agents. It is estimated that several hundred thousand different chemicals entities are present in the environment. It is clear that an extensive number of these have not yet been assayed for carcinogenicity. It is important to public health that such information be obtained. It is anticipated that the results from this program may lead to the prevention or reduction of the unintentional exposure of man to carcinogenic materials.

Proposed Course of Project: Many chemicals have reached the end of their testing phase resulting in a heavy workload in pathology, data analysis and reporting. However, mechanisms and personnel have been mustered to meet the requirement that all reports on over 200 compounds be completed early during the next fiscal year.

Additional studies are needed to improve the methodology of testing. Research is needed to better predict a tolerated level for long-term treatment. Additional studies are planned to assess species/strain sensitivity to allow better selection of the test animal. Pharmacokinetics and metabolic studies are planned in order to increase the predictability of the bioassays. It is essential that the bioassay address not only the question of whether a chemical is or is not carcinogenic but also the question as to what carcinogenic risk the chemical may present to humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CP-04699-08-CBPR

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogenesis Bioassay Data System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. C. Chu, Chemist	CBPR NCI
Other:	M. S. Linhart, Computer Programmer	CBPR NCI
	S. Siegel, Research Biologist	CBPR NCI
	J. W. Chase, Technical Information Sp.	CBPR NCI
	U. N. Evans, Computer Technician	CBPR NCI

COOPERATING UNITS (if any)

Data Management Branch, Division of Computer Research and Technology, NIH, Field Studies and Statistics Program, NCI

LAB/BRANCH

Carcinogen Bioassay and Program Resources Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.00	2.00	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Carcinogen Bioassay Data System is used to collect, monitor, store, analyze and report experimental data and results from bioassay operations. It is now in full operation with new bioassay experiments being entered as they are initiated. In the past year, over 250,000 data collection forms were processed and entered into the CBDS data base. During this period, special attention has been given to generating reports and analyzing data. For each compound in the system, there is data on experimental design, an individual animal tumor pathology table and tumor incidence summary table, individual animal non-tumor pathology and tissue count table, survival curve analysis, animal weight curves, and statistical analysis of all lesions occurring at an incidence above 5%. Reports are in the process of being generated for over 200 compounds. Statistical analysis of survival and specific tumor types was established using unadjusted and time-adjusted techniques. The unadjusted methods include the Armitage and Cox test for linear trend and Fisher-Irwin Exact test while the time-adjusted methods involve the Cox-Tarone test for trend. In addition, chemical and bioassay contract information sub-systems are maintained.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To develop, implement, maintain and modify, when needed, a comprehensive information system for collection, storage, manipulation, retrieval, analysis and reporting of data pertaining to carcinogenesis biaossay studies. In so doing, to create and maintain a file of data about (a) substances on test in the Carcinogenesis Program, (b) experiments and related experimental design in bioassay studies, (c) diagnoses of lesions from suspected carcinogenic agents on test, and corresponding control groups.

Methods Employed: The development and operation of the CBDS System involves standard taxonomy, systems-operations, computer programming, data retrieval systems and statistical analysis utilizing the NIH/DCRT hardware and software environment. The Carcinogenesis Bioassay Data System comprises four basic subsystems which are described below:

(1) Bioassay Contractor Subsystem - The contractor scientists document the design and results of experiments on a series of standard forms for the NCI and submit these forms to the Operations Subsystem.

(2) Operations Subsystem - An operations contractor provides and obtains completed forms from the bioassay contractors, checks them for accuracy and completeness, logs their progress through the system, microfilms them, keypunches, key-verifies input documents corresponding to the data forms, and submits computer jobs on new or replacement data for entry into the CBDS data base. This system also maintains the CBDS data base and auxiliary bioassay information files such as chemical and contractor information.

(3) Reporting Subsystem - The computer systems manager and programmers design, develop and implement computer programs to generate preliminary management and scientific output reports from the CBDS data base.

(4) Statistical Analysis Subsystem - Computer programmers and statisticians teaming together have implemented statistical routines for analyzing chronic toxicity and carcinogenicity data of CBDS animals. These methods include Kaplan-Meier Survival Curves, Cox-Tarone Test for trend, Armitage Test for trend and the Fisher-Irwin Exact Test.

Major Findings: In the past year, many experiments have come to completion and statistical analysis of survival and tumor incidence data have been developed to pin-point statistically significant results. Tables have been computer generated to report tumor incidence in a summary form as well as for individual animals. Capabilities for graphing of survival, tumor incidence, animal weights and food consumption have been developed. Statistical analysis are performed on all lesions occurring 5% or more in a group and the results are reported in tabular form. Then these data are assembled for each compound in the system for report writing.

Significance to Biomedical Research and the Program of the Institute:

Because of the interest of other governmental agencies and the public in the Carcinogenesis Bioassay Program, it is essential that data and administrative information be quickly processed, accurate and easily available. The CBDS provides this capability, and thus contributes uniformity and completeness to reporting and analyzing results of bioassay studies, and provides standardization of chemical and tumor nomenclature.

Proposed Course of Project: In Fiscal 1978, it is estimated that this project will generate reports on 45-60 chemicals, while incorporating information on 20-30 new bioassay chemicals into the CBDS data base. Additional statistical methods for analyzing the bioassay results will be explored. Emphasis will also be placed on increasing the efficiency of report generations.

A redesign of the system presently underway will be addressing problems associated with increasing the level of automated data processing in the system.

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY BRANCH

July 1, 1976 through September 30, 1977

"Plans, develops, and conducts a research program including (1) identification, chemical analysis, mode of formation, and metabolism of various classes of chemical carcinogens; (2) studies on the toxicology and metabolic pathways of carcinogens in selected animal systems and in relation to man; (3) correlation of chemical and toxicological data for the selection and screening of chemical carcinogens; and (4) development of biological and biochemical methods for the identification of reactive carcinogenic metabolites."

The Carcinogen Metabolism and Toxicology Branch (CMT) is involved both in a number of in-house research projects and in the scientific direction of collaborative projects through different areas of the Carcinogenesis Program.

The collaborative projects are conducted through the contract segment which coordinates contracts on the toxicology and metabolism of carcinogens (aromatic amines, some halogenated aliphatic compounds, aflatoxin and derivatives), and part of the program on nitrosamines. Branch staff are involved in several projects on nitrosamines, and in the direction of the repository of chemical carcinogens. Branch staff are also involved in the selection of compounds for the bioassay program and are involved in the Bioassay Program Review. Staff of the Branch in addition act as monitors for one of the CREG programs. Various Branch staff are highly involved with the program at the FCRC as project monitors and coordinators. Furthermore, Branch members are consultants for various national and international activities in the areas of chemical carcinogenesis and environmental carcinogens.

The research activities in the Office of the Chief are directed largely towards studies on the metabolism and activation of various classes of chemical carcinogens and studies on the role of certain nutritional factors in the mechanism of action of chemical carcinogens. In addition, some small scale bioassays in support of studies on structure-activity relationships are being conducted.

Further investigations on the metabolism of the environmental material 2,4-toluenediamine, an intermediate for polyurethane foam, are continuing. Some possible metabolites such as benzyl alcohol derived from 2,4-toluenediamine or possible N-hydroxy derivatives of some of the aminotoluenes may be involved. In addition, the metabolism of 2,4-toluenediamine in liver microsomes or in liver cytosol is being conducted to collaborate or confirm the results from the in vivo experiments.

The investigation on the metabolism of 2-aminoanthraquinone in animals is continuing. There is a possibility that in addition to N-acetylation of the amine, N-formylation may occur. This hitherto unknown reaction may be more general than thought possible from previous work on metabolism of aromatic amines.

A new investigation begun during the past year has been a study of 2,4-diaminoanisole, a very mutagenic compound used in hair dye preparations. The experiments conducted so far in rats showed that the compound was excreted rapidly and that acetylation of the amino groups, hydroxylation on the ring and to a minor extent demethylation had occurred. An investigation on the metabolism of this compound in in vitro systems is also being conducted.

It was shown that the commonly used antioxidant BHT inhibited the effect of a strong intestinal carcinogen in rats when BHT was given simultaneously with the carcinogen. If BHT was given after the treatment with carcinogen, the net result was to enhance the carcinogenic action.

In other work on nutritional problems in carcinogenesis, it was found that L-tryptophan tended to reduce the carcinogenic effect of diethylnitrosamine and 4-dimethylaminoazobenzene. Further studies on the mechanism of action are warranted.

Results from some of the small scale bioassays for structure-activity relationships have revealed that a very interesting compound provided by Dr. C. Michejda induces stomach adenomatosis with a very high incidence. This compound may prove to be a useful model compound for studies of this condition.

Analytical Chemistry Section - *"Conducts independent research on the organic, analytical, and biological chemistry of nitrosamines and related compounds and collaborates with other members of the Carcinogenesis staff in matters requiring chemical expertise, especially in problems of identifying non-polymeric organic molecules."* Activities for the past year have focused on two main areas:

N-Nitroso Compounds. (a) An improved synthesis of butyl(carboxypropyl)-nitrosamine has been developed, and the product has been shown, in collaboration with Dr. D. Reese and colleagues, to induce focal suppression of hyperplasia in organ cultured rat bladder epithelium. (b) Highly purified samples of ^{14}C-dimethylnitrosamine-d_6 and Z-methyl(methyl-d_3)nitrosamine have been synthesized and are being used to probe mechanisms of the interaction of dimethylnitrosamine with cellular components. (c) In collaboration with Dr. J. Rice and colleagues, methyl(acetoxymethyl)nitrosamine has been shown to be a potent intestinal carcinogen in rats on intraperitoneal injection. This highly interesting compound and certain derivatives thereof are now under study in several other model systems. (d) A variety of lipid soluble metal nitrites and nitrosyls, including one compound with a possible dioxygen ligand, have been synthesized and shown to be effective nitrosating agents in nonaqueous media. (e) High yields of carcinogenic nitrosamines

have been observed to form at room temperature during exposure of methylene chloride solutions of the secondary amines to solid sodium nitrite. (f) Chemical ionization mass spectrometry has been used to study protonation and fragmentation of several nitrosamines.

Mass Spectrometry. The mass spectrometry laboratory is involved in carrying out independent research as well as participation in collaborative projects with a number of investigators on carcinogenesis related problems, where mass spectrometry, as an analytical tool, can be applied.--(a) Five C-α-acetoxylated dialkyl nitrosamines, synthesized in our laboratory, were found to exhibit novel electron impact induced acyl migrations, presumably to the nitrosamino moiety. The unimolecular gas phase decomposition pathways of these biologically interesting compounds may shed light upon their chemical reactivities and their structural properties. (b) In supporting the projects within the Branch, mass spectral measurements on a number of synthetic intermediates and on in vivo metabolites from 2,4-diaminotoluene, 2,4-diaminoanisole, and 2-aminoanthraquinone have facilitated chemical structure determinations. (c) In collaborative work with the Chemistry Branch of the Division, chemical studies and mass spectra on the acetonide derivatives of four isomeric 7, 8, 9, 10-tetrahydroxy-tetrahydrobenzo[a]pyrenes allowed their unambiguous structural determination, the tetrols being solvolysis products of the metabolically important, but chemically unstable 7,8-diol-9,10-epoxide derivatives of B[a]P. Stable isotope labeling studies provided mechanistic information on the course of B[a]P metabolism. (d) In a continuing collaborative investigation with Dr. R. E. Moore of the University of Hawaii on the chemical components of the edible seaweed Asparagopsis taxiformis (Rhodophita), more than 80 multiple halogenated (chlorinated, brominated, iodinated) C_1-C_4 compounds have been structurally determined. More recently identified structural types include halogenated but-3-en-2-ols, acetic acids and acrylic acids. A number of these naturally occurring compounds could be viewed as potential carcinogens. (e) In a collaborative effort with the Fermentation Laboratory, FCRC, on studies of potential chemotherapeutic agents, detailed mass spectral analysis has been completed on a number of daunorubicin derivatives, and the results applied to the identification of daunorubicin metabolites. The mass spectral analysis of rifamycin disclosed some characteristic fragmentations applicable to the identification of unknown homologs. (f) Efforts are being continued in the analysis of bioactive materials that may play a role in cancer causation and prevention mechanisms, such as vitamin A derivatives (collaborators Dr. L. M. DeLuca and independently Dr. M. Sporn), and substances with immunological properties, e.g. multiacylated trehalose-2-sulfate derivatives (collaborator Dr. M. B. Goren).

Nutrition and Metabolism Section - *"Plans, develops and conducts research on (1) the effects of dietary constituents on the activities of various classes of chemical reactivities of ultimate carcinogens; (2) the metabolism of dietary components known to modify the carcinogenic process; and (3) the correlation of chemical and toxicological data for the identification of chemical carcinogens."* The section has focused its interest on the role of the lipotropes methionine, choline, vitamin B_{12} and folic acid in chemical carcinogenesis. Chronic administration of diethylnitrosamine (DEN) to rats

produced significant hepatic methyl and folate deficiencies. The deficiencies were characterized by low hepatic levels of active folates and S-adenosyl-methionine and by an altered distribution of cobalamin cofactors. The increased synthesis of phosphatidyl choline seen in the DEN-treated rats was a likely cause of the lipotrope deficiency. Vitamin B_{12} deficiency appeared to be slightly protective against the carcinogenic activities of diethyl-nitrosamine, azoxymethane, and N-methyl-N-nitroso-N'-nitroguanidine in rats but had no significant effect on five other carcinogens. Even in the absence of a methyl deficiency, methionine and choline reversed the folate deficiency of rapidly proliferating liver.

The growth of transformed rat liver epithelial cells is markedly inhibited by the replacement of methionine by homocysteine in the medium; normal liver epithelial cells are much less affected by the change. The elevated methionine requirement by transformed cells could not be attributed to an increased methylation of phosphatidyl ethanolamine to phosphatidyl choline in these cells. The evidence accumulated to date suggests the following generalizations between lipotropes and carcinogenesis: (a) The accelerating action of methyl-deficient diets on carcinogenesis appears to be due to their effects on carcinogen metabolism. (b) The previously reported acceleration of chemical carcinogenesis by vitamin B_{12} may be ascribed to the host's synthesis of methionine to meet the demands of the tumor: This B_{12} effect is thus lost in methionine-adequate animals. (c) There is an elevated requirement for the methyl donors and for reduced folate in rapidly proliferating tissue.

The Section's research activities have also included an examination of species, sex and strain sensitivities to the formation and inhibition of tumors and investigations of structure-activity relations in chemical mutagenesis and carcinogenesis.

Equimolar levels of dimethylhydrazine and azoxymethane produced colon tumors in male F-344 rats; such tumors were seen in female F-344 rats treated with azoxymethane, but not with dimethylhydrazine. The mutagenic activities of alkyl halides with polar side groups towards Salmonella and E. coli WP2 (hcr^-) were determined and compared with their carcinogenic activities toward mouse lung. The compounds were mutagenic but were too toxic to be adequately tested in vivo.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04500-06-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Appearance of α-Fetoproteins (AFP) in the Serum of Animals Given Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. K. Weisburger	Chief	CMT NCI
OTHER:	T. Benjamin	Chemist	CMT NCI

COOPERATING UNITS (if any)
Dr. Stewart Sell, University of California, San Diego

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rat or mouse antiserum is raised in rabbits; the antiserum is purified to obtain antiserum specific to alpha-fetoprotein (AFP). Other mice or rats are treated with various classes of chemical carcinogens known to induce tumors of the liver and other organs. Sera are obtained at intervals from these animals for radioimmunoassay. Positive results indicate that AFP has been released by the cells into the serum.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: A carcinoembryonic plasma protein, α-fetoprotein (AFP), has been found in the serum of some patients with hepatocellular carcinoma and a few other lesions such as teratomas. Depending on geographic location, the percentage of AFP-positive patients varies from 30% in some areas like the United States or Great Britain, to 80% in Africa. The presence of AFP may be a diagnostic marker for hepatocellular carcinomas. A number of questions on the mechanisms of formation and of the derepression of this fetal protein are of interest in relation to the etiology of carcinoma in man.

Methods Employed: Utilizing amniotic fluid of inbred female Fischer strain rats or C57Bl/6 mice at 16 to 20 days of gestation as antigen, antiserum was raised in rabbits. The antiserum was purified by specific techniques to secure an AFP-specific antiserum. Radioimmunoassays of selected serum samples are performed by Dr. S. Sell, University of California, San Diego.

Major Findings: Lack of α-fetoprotein (AFP) in serum of mice given a single injection of N-nitrosobutylurea. Swiss mice were injected with a single dose of nitrosobutylurea, a treatment which we have shown to lead to a high incidence of intestinal tumors. The mice were bled at periodic intervals and the sera were sent to Dr. Stewart Sell. Over a 50 week period, even when some of the mice obviously were beginning to show tumors, the sera did not show an elevation of AFP levels. It is evident that AFP may be very specific for hepatotoxins or hepatocarcinogens and may not be evident in tumors at other organs.

Significance to Biomedical Research and the Program of the Institute: The current test series was initiated because of the differences in the presence of a diagnostic antigen AFP in the serum of human patients with liver cancer. The etiology of liver cancer in different parts of the world is unknown but presumably rests on a variety of inciting factors and chemical carcinogens. Studies on physiologic conditions and specific carcinogenic agents which would produce AFP and others which would not, might give clues to etiologic factors operating in various regions. An understanding of the factors controlling the release of a fetal antigen from normal or cancer cells might further our knowledge of the mechanisms of carcinogenesis. Eventually it may lead to information which can be used to control and perhaps prevent cancer development. Studies on the effect of immunocompetence in the carcinogenic process likewise bear on these same problems. Their resolution would augment means of controlling the progress of the disease.

Proposed Course of Project: In view of the negative results this project is being concluded.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04510-04-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Environmental and Genetic Factors in Digestive System Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Richard S. Yamamoto Research Chemist CMT NCI
OTHER: None

COOPERATING UNITS (if any)
Dr. T. Sugimura, Japanese National Cancer Center Research Inst., Tokyo
Dr. T. Matsushima, Institute of Med. Sciences, University of Tokyo
Dr. M. Levitt, Research Pathologist, Experimental Pathology Branch, NCI

LAB/BRANCH
Carcinogen Metabolism & Toxicology Branch, Carcinogenesis Program

SECTION
Nutrition & Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland, 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.75	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study of strain and sex susceptibility to colon carcinogenesis using 1,2-dimethylhydrazine (DMN) and azoxymethane (AOM) in mice and rats. Difference in response to the different carcinogens was found in the sexes in DMH but not in AOM. Resistant strains developed tumors in other organs. 2,2'-Dihydroxypropane-nitrosamine (DIPN) produced pancreatic duct tumors in Syrian golden hamsters on various levels of dietary protein and fats.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine interrelations of environmental and genetic factors in the development of gastrointestinal tract cancer. Epidemiologic studies show that dietary factors play an important role as cofactors in digestive tract carcinogenesis.

Methods Employed: Various strains of rats and mice are compared for their susceptibility to DMH and AOM carcinogenesis. Tumors and other physiological changes are observed. 2,2'-Dihydroxypropane-nitrosamine (DIPN) is used to produce pancreatic duct carcinoma in hamsters on various different dietary levels of fat and protein.

Major Findings: 1) Response of different strains of mice to AOM and DMH. DMH, at levels of 0.2 mmole per kg mouse once a week for 10 weeks did not produce colon tumors in female C57BL/6N strain mice in 40 weeks, but a few tumors did appear at 60 weeks. However, this level was very effective in male C57BL/6N mice. Both sexes responded to AOM tumorigenesis at this level.

2) Sex differences in response of rats to DMH and AOM colon carcinogenesis. Female F344 rats were resistant to DMH colon carcinogenesis, whereas the male F344 rats readily produced colon and small intestinal tumors. However, with both male and female F344 rats, AOM induced colon tumors. All the female rats on AOM also developed kidney tumors.

3) Response of hamsters to pancreatic duct carcinogenesis on semi-purified diet. The Syrian golden hamsters receiving a low dose of 2,2'-dihydroxypropane-nitrosamine responded to different dietary levels of protein and fat.

Significance to Biomedical Research and the Program of the Institute: The high morbidity and mortality from gastrointestinal cancer is a major health problem in the United States. The development of animal models of gastrointestinal neoplasms, and their use for the study of etiological factors which act in combination of factors which modify the incidences and development of such tumors, is expected to lead to knowledge relevant to their prevention.

Proposed Course of Project: Further studies of dietary ingredients, antioxidants, antibiotics and enzyme inhibitors will be made in conjunction with compounds known to produce gastrointestinal tract cancer. Resistant vs. susceptible strains and species will be used to study the metabolic activation of the colon carcinogens. The influence of dietary components on pancreatic carcinogenesis by DIPN will be concluded.

Publications

Yamamoto, R. S. and Weisburger, E. K.: The role of hormones on digestive and urinary tract carcinogenesis. Recent Prog. Horm. Res. 33: 617-653, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04542-05-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemistry of N-Nitroso Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Keefer	Head, Anal. Chem. Sec.	CMT NCI
OTHER:	P. Roller	Chemist	CMT NCI
	R. Angeles	Staff Fellow	CMT NCI
	J. Miller	Chemist	CMT NCI
	S. Uhm	Visiting Fellow	CMT NCI
	C. Michejda	Guest Worker	CMT NCI
	J. Rice	Head, Perinatal Carcinogenesis Sec.	EXP NCI
	D. Reese	Senior Staff Fellow	LC NCI
	R. Friedman	Biochemist	LC NCI
	W. Lijinsky	Litton Bionetics	FCRC
	W. Palmer	Litton Bionetics	FCRC

(continued on next page)

COOPERATING UNITS (if any)

Perinatal Carcinogenesis Section, EXP, NCI
Lung Cancer Branch, NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION
Analytical Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data concerning the chemical and physical properties of the carcinogenic N-nitroso compounds will be collected. Specific emphases will include studies of: a) promotion of N-nitrosation reactions of environmental interest by metal species; b) stereochemistry of oxidative attack by microsomes upon dimethylnitrosamine molecules; c) studies of tautomerism in the conjugate acid of the polydentate nitrosamino ligand; d) preparation of novel nitrosamines and their derivatives for chemical and biological studies. Possible implications of this work with respect to the overall goal of human cancer prevention will be considered.

PHS-6040
(Rev. 10-76)

W. Gaffield	USDA, Berkeley, CA	
S. Joshi	Staff Fellow	EXP NCI
M. Kroeger-Koepke	Summer Employee	CMT NCI
N. Davidson	Summer Employee	CMT NCI
M. Wenk	Microbiological Assoc.	
H. Taylor	Oak Ridge	
P. Swann	Courtauld Inst., London	

Project Description

Objectives: (1) To investigate mechanisms by which metal ions might promote the formation of N-nitroso compounds in the environment. (2) To study mechanisms of metabolism and carcinogenesis by N-nitroso compounds using deuterium labeled substrates. (3) To synthesize models of possible metabolites of certain N-nitroso compounds for comparisons of their physical, chemical and biological properties with those of the parent procarcinogens. (4) To study mechanisms of possible artefact formation during analysis for environmental N-nitroso compounds.

Methods Employed: The standard methods of synthetic and mechanistic chemistry have been used in these investigations.

Major Findings: An improved synthesis of butyl(carboxypropyl)nitrosamine has been developed, and the product has been shown in collaboration with Dr. D. Reese and colleagues to induce focal suppression of hyperplasia in organ cultured rat bladder epithelium. Highly purified samples of ^{14}C-dimethylnitrosamine-d_6 and 2-methyl(methyl-d_3)nitrosamine have been synthesized and are being used to probe mechanisms of dimethylnitrosamine's interaction with cellular components. In collaboration with Dr. J. Rice and colleagues, methyl(acetoxymethyl)nitrosamine has been shown to be a potent intestinal carcinogen in rats, and this highly interesting compound and certain derivatives thereof are now under study in several other model systems. A variety of lipid-soluble metal nitrites and nitrosyls, including one compound with a possible dioxygen ligand, have been synthesized and shown to be effective nitrosating agents in nonaqueous media. High yields of carcinogenic nitrosamines have been observed to form at room temperature during exposure of methylene chloride solutions of the secondary amines to solid sodium nitrite.

Significance to Biomedical Research and the Program of the Institute: With considerable public attention focused on the possibility that N-nitroso compounds might be responsible for some human cancer, collection of data on the chemical, physical, and biological properties of this hitherto poorly understood class of compounds is an urgent matter. Certain aspects of the studies reported here may aid in developing methods for predicting and

analyzing the environmental distribution of these potential carcinogens, while other aspects are aimed at shedding light on the biochemical mechanisms by which these compounds exhibit their untoward effects.

Proposed Course of Project: The studies described above will be completed and published, and the important hypotheses and implications they suggest will be investigated as appropriate.

Publications

Keefer, L. K.: Promotion of N-nitrosation reactions by metal complexes. Environmental N-Nitroso Compounds Analysis and Formation, IARC Scientific Publications, No. 14, 153-159, 1976.

Michejda, C. J., Davidson, N. and Keefer, L. K.: Photochemical perturbation of Z ⇌ E equilibria in nitrosamines. J. C. S. Chem. Comm. 16: 633, 1976.

Lijinsky, W., Taylor, H. W. and Keefer, L. K.: Reduction of rat liver carcinogenicity of nitrosomorpholine by α-deuterium substitution. J. Natl. Cancer Inst. 57: 1311-1313, 1976.

Joshi, S. R., Rice, J. M., Wenk, M. L., Roller, P. P. and Keefer, L. K.: Brief Communication: Selective induction of intestinal tumors in rats by methyl(acetoxymethyl)nitrosamine, an ester of the presumed reactive metabolite of dimethylnitrosamine. J. Natl. Cancer Inst. 58: 1531-1536, 1977.

Gaffield, W., Roller, P. P., Palmer, W. G. and Keefer, L. K.: Tritium gas exposure as an alternative to base-catalyzed exchange for the one-step tritiation of nitrosamines. Journal of Labelled Compounds and Radiopharmaceuticals (in press).

Gaffield, W., Keefer, L. K. and Roller, P. P.: Synthesis of the selective bladder carcinogen, N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN). Organic Preparations and Procedures International (in press).

Gaffield, W., Fish, R. H., Holmstead, R. L., Poppiti, J. and Yergey, A. L.: Chemical ionization-mass spectrometry of nitrosamines. Environmental N-Nitroso Compounds Analysis and Formation, IARC Scientific Publications, No. 14, 11-20, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04580-03-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of Lipotropes in Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lionel A. Poirier	Head, Nutrition & Metabolism Section	CMT NCI
OTHER:	Mary Wilson	Chemist	CMT NCI
	H. J. Raj	Visiting Fellow	CMT NCI
	J. Leitzer	Jr. Asst. Surgeon	CMT NCI
	W. Anderson	Physical Science Aid	CMT NCI

COOPERATING UNITS (if any) Dr. E. L. R. Stokstad, University of California, Berkeley
Dr. Adrianne Rogers, Massachusetts Institute of Technology
Dr. J. C. Linnell, Westminster Square Hospital, London
Dr. J. Farrell, Experimental Pathology Laboratories

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION
Nutrition and Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms behind the alteration of chemical carcinogenesis by the dietary lipotropes, choline and vitamin B_{12} have been studied. The activation of the carcinogen 2-acetylaminofluorene was increased in rats fed a low lipotrope diet, which also increased its carcinogenic activity. The chronic administration of diethylnitrosamine (DEN) to rats led to an increased uptake of labelled precursors into hepatic lecithin; the uptake of labelled precursors into phosphatidyl ethanolamine and phosphatidyl serine was unaffected by chronic DEN administration. Transformed liver epithelial cells were found to have a higher requirement for methionine than normal liver epithelial cells. Mice bearing leukemia L-1210 survived slightly longer on methionine-deficient diets than on methionine-adequate diets. In rapidly proliferating liver methionine reversed the folate-deficiency apparently caused by a high ratio of thymidylate synthetase to dihydrofolate reductase.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: Folic acid and the dietary lipotropes methionine, choline, and vitamin B_{12} significantly modify the production of tumors by certain chemical carcinogens. The extent of and the mechanisms behind these effects are being investigated. The effects of the lipotropes on chemical carcinogenesis by a variety of agents are under investigation. In addition, the mutual metabolic interactions of the lipotropes and the chemical carcinogens are to be determined.

Methods Employed: The carcinogenic activities of several standard carcinogens were compared in rats fed a vitamin B_{12}-deficient diet and the same diet supplemented with 100 μg/kg of vitamin B_{12}. S-Adenosylmethionine was determined using isotope-dilution techniques and thin layer chromatography. The urinary metabolites of AAF were measured by conventional paper chromatography, supplemented with high pressure liquid chromatography. The folate and cobalamin cofactors were separated by thin layer and column chromatography followed by microbiological assay. The incorporation of labelled one-carbon precursors into the phospholipids of DEN-treated rats was measured by standard extraction, colorimetric and chromatographic techniques. The nutritional requirements of normal and transformed liver epithelial cells were determined in tissue culture systems developed in this laboratory. The survival of mice treated with L-1210 leukemia cells and fed amino acid-defined diets were determined by standard protocols developed by DCT.

Major Findings: The chronic administration of DEN to rats led to hepatic lipotrope deficiencies. These were marked by decreased hepatic levels of active folates, and of S-adenosylmethionine, and of total hepatic content of vitamin B_{12}. Further, chronic DEN administration led to an increased uptake of the methyl groups of methionine and choline into phosphatidyl choline, but did not affect the uptake of precursors into phosphatidyl ethanolamine and phosphatidyl serine. Rats fed a vitamin B_{12} -deficient diet and treated with DEN developed tumors more slowly than DEN-treated rats fed the same diet supplemented with vitamin B_{12}. Transformed liver cell lines were found to have a higher requirement for methionine than did normal liver cell lines. On the other hand, lipotrope deficiency was found to increase the ratio of N-hydroxylation to ring hydroxylation of 2-acetylaminofluorene in male rats fed a low lipotrope diet that increased the activity of this carcinogen. Methionine reversed the folate deficiency associated with rapidly proliferating liver without altering the hepatic levels of S-adenosylmethionine. Thus, the lipotropes appear to modify chemical carcinogenesis by altering carcinogen metabolism and tumor nutrition.

Significance to Biomedical Research and the Program of the Institute: The experiments under this project aim to delineate the roles of choline, methionine, vitamin B_{12} and folic acid in chemical carcinogenesis. The evidence accumulated to date suggests the following partial generalizations: 1) The accelerating effect of low-lipotrope diets on carcinogenesis appears attributable to their affects in carcinogen metabolism. 2) The accelerating affects of vitamin B_{12} on chemical carcinogenesis may be due to the host's synthesis of methionine to meet the demands of the tumor. 3) The lipotrope deficiencies seen in carcinogen-treated or in tumor-bearing animals appears to be a consequence of an elevated requirement for methionine, choline and reduced folates in rapidly proliferating tissue. It is hoped to use these dietary factors in the prevention or inhibition of human cancer.

Proposed Course of Project: Essentially the project will continue along the same lines. The effect of a combined vitamin B_{12} and methionine deficiency on chemical carcinogenesis are being investigated. The cause of the elevated methionine requirement of transformed cells will be determined. Phospholipid biosynthesis in normal and transformed liver cells will be compared.

Publications

Poirier, L. A. and Boutwell, R. K.: Current problems in nutrition and cancer. An introduction. Federation Proc. 35: 1307-1308, 1976.

Buehring, Y.S.S., Poirier, L. A. and Stokstad, E. L. R.: Folate deficiency in the livers of diethylnitrosamine-treated rats. Cancer Res. 36: 2775-2779, 1976.

Linnell, J. C., Quadros, E. V., Matthews, D. M., Morris, H. P. and Poirier, L. A.: Altered cobalamin distribution in rat hepatomas and in the livers of rats treated with diethylnitrosamine. Cancer Res. (in press).

Petri, W. A. and Poirier, L. A.: A methionine-reversible folate deficiency in rats following the acute administration of diethylnitrosamine and ∂-naphthylisothiocyanate. Chem.-Biol. Interactions (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04581-02-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Analytical Applications of Mass Spectrometry to Carcinogenesis Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Roller	Chemist	CMT NCI
OTHER:	L. Keefer	Head, Anal. Chem. Sec.	CMT NCI
	J. Miller	Chemist	CMT NCI
	R. Angeles	Staff Fellow	CMT NCI
	J. Rice	Head, Perinatal Carcinogenesis Sec.	EXP NCI
	L. DeLuca	Head, Differentiation Control Sec.	EXP NCI
	H. Gelboin	Branch Chief	CH NCI
	S. Yang	Senior Staff Fellow	CH NCI
	A. Aszalos	Litton Bionetics	FCRC
	J. Chan	Litton Bionetics	FCRC
	R. Moore	Chem. Dept.,U. Hawaii, Honolulu	
	M. Goren	Natl. Jewish Hospital, Denver, Colo.	

COOPERATING UNITS (if any)
Molecular Carcinogenesis Section, CH, NCI
Perinatal Carcinogenesis Section, EXP, NCI
Differentiation Control Section, EXP, NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION
Analytical Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The laboratory is involved in carrying out independent research, as well as participation in collaborative projects on carcinogenesis related problems, where mass spectrometry, as an analytical tool, can be applied to determine the structure or confirm the identity of non-polymeric organic molecules of interest. Studies include: (1) the identification of carcinogen metabolites in activation and metabolism studies; (2) the identification of naturally occurring potential carcinogens in the environment; (3) elucidation of mass spectral fragmentation mechanisms; (4) development of methods for derivatization and analysis of carcinogens as well as of antitumor agents; (5) analysis of bioactive materials that may play a role in cancer causation and prevention mechanisms, such as vitamin A derivatives and substances with immunological properties.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To apply the mass spectrometry method for the analysis and identification of metabolites in carcinogen activation and metabolism studies. (2) To study in some detail the mass spectra of carcinogens and of some of their possible metabolites, such as aromatic hydrocarbons and N-nitroso compounds, with the aim of applying this knowledge to develop appropriate analytical methods. (3) To analyze the natural components, alkyl, and vinyl halides in certain edible sea-weeds, which may pose possible carcinogenic hazard to people ingesting them. (4) To elucidate the chemical structure of certain natural bioactive materials that may play a role in cancer causation and prevention mechanisms.

Methods Employed: A high resolution double focusing mass spectrometer system was used, interfaced with a gas chromatograph, and run in the electron impact ionization mode. A number of synthetic and metabolic samples were also prepurified by collaborators using high pressure liquid chromatography. In some cases suitable chemical characterizations were necessary. A number of N-nitroso-C-α-acetoxydialkylamines were synthesized for mass spectral fragmentation studies.

Major Findings: Data are being collected on the mass spectra of N-nitroso compounds, a class of strong animal carcinogens, from spectra run in our laboratory, as well as from the literature. The mass spectral fragmentation mode of a set of five synthetic N-nitroso-C-α-acetoxydialkylnitrosamines were examined and were found, among other features, to demonstrate novel acyl migrations upon electron impact with concomitant loss of the alkyl aldehyde moiety.

The mass spectra of a large number of benzo[a]pyrene derivatives are being studied, both of synthetic as well as of metabolic origin, including those labelled with ^{18}O, ^{2}H, and ^{14}C isotopes. In a collaborative effort with the Chemistry Branch of the Division (Project Number Z01-CP-04597-02-CH), chemical studies and mass spectra on the six acetonide derivatives of four isomeric 7,8,9,10-tetrahydroxytetrahydrobenzo[a]pyrenes allowed their unambiguous structural determination, the tetrols being solvolysis products of the metabolically important, but chemically unstable 7,8-diol-9,10-epoxide derivatives of B[a]P. ^{18}O-Labelling studies provided information on the metabolic pathway, B[a]P to 7,8-epoxide, to 7,8-diol, to diol-epoxide, and also on formation of other diols and of phenols.

In collaborating with R. E.Moore of the University of Hawaii in a continuing investigation on the chemical components of the edible seaweed *Asparagopsis taxiformis* (Rhodophita and Delile), more than 80 multiply halogenated (chlorinated, brominated, iodinated) C_1 - C_4 compounds have been structurally determined. More recently identified structural types include tri-, tetra-, and pentahalobut-3-en-2-ols, mono-, and dihaloacetic acids, and mono-, di-, and trihaloacrylic acids. A number of these naturally occurring compounds could be viewed as potential carcinogens.

In a collaborative effort with the Fermentation Laboratory, FCRC (Project 17, A. Aszalos), on studies of potential chemotherapeutic agents, detailed mass spectral analysis has been completed on a number of duanorubicin derivatives, and the results applied to the identification of daunorubicin metabolites. The mass spectral analysis of peracetylated and of pertrifluoroacetylated rifamycin SV disclosed some characteristic fragmentations applicable to the identification of unknown homologs.

In collaboration with M. B. Goren of the National Jewish Hospital, Denver, joint mass spectral studies were instrumental in structural elucidation of multiacylated trehalose-2-sulfates and of their closely related homologs, all biologically important constituents of *Mycobacterium tuberculosis*. These compounds may have interesting immunological properties and perhaps some tumor suppressive activity.

Significance to Biomedical Research and the Program of the Institute: The understanding of the mass spectral behavior of specific classes of organic compounds is prerequisite to the interpretation of spectra and to the structural determination of a number of biologically important compounds. Toward this goal detailed spectral studies on known N-nitroso compounds, polycyclic aromatic compounds and on anthracycline- and macrocyclic antibiotics have been valuable. Structural studies on metabolites of aromatic amines and of polycyclic aromatic hydrocarbons have given insight into the pathways of carcinogen degradation or activation. Identification of carcinogens or of potential carcinogens in man's immediate environment or food staple is important in developing prevention methods.

Proposed Course of Project: The studies described above will be completed and published. A number of metabolic studies are continuing and their significance to cancer causation will be evaluated.

Publications

Burreson, B. J., Moore, R. E. and Roller, P. P.: Volatile halogen compounds in the alga *Asparagopsis taxiformis* (Rhodophyta). J. Agric. Food Chem. 24: 856-861, 1976.

Goren, M. B., Brokl, O., Roller, P. P., Fales, H. M. and Das, B. C.: Sulfatides of *Mycobacterium tuberculosis:* the structure of the principal sulfatide (SL-I). Biochemistry 15: 2728-2735, 1976.

McCaustland, D. J., Fischer, D. L., Kolwyck, K. C., Duncan, W. P., Wiley, Jr., J. C., Menon, C. S., Engel, J. F., Selkirk, J. K. and Roller, P. P.: Polycyclic aromatic hydrocarbon derivatives: synthesis and physicochemical chacterization, in Carcinogenesis, Vol. 1, Polycyclic Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis, eds. Freudenthal, R. I. and Jones, P. W., Raven Press, New York, 1976, 349-411.

Roller, P. P., Sutphin, M. and Aszalos, A. A.: Mass spectrometry of N-acylated daunorubicin derivatives. Biomed. Mass Spectrometry 3: 166-171, 1976.

Woolard, F. S., Moore, R. E. and Roller, P. P.: Halogenated acetamides, but-3-en-2-ols, and isopropanols from *Asparagopsis taxiformis*(delile)*trev.* Tetrahedron 32: 2843-2846, 1976

Yang, S. K., McCourt, D. W., Roller, P. P. and Gelboin, H. V.: Enzymatic conversion of benzo[a]pyrene leading predominantly to the diol-epoxide r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenz[a]pyrene through a single enantiomer of r-7,t-8-dihydroxy-7,8-dihydrobenzo[a]pyrene. Proc. Natl. Acad. Sci. USA 73: 2594-2598, 1976.

Aszalos, A. A., Bachur, N. R., Hamilton, B. K., Langlykke, A. F., Roller, P. P., Sheikh, M. Y., Sutphin, M. S., Thomas, M. C., Wareheim, D. A. and Wright, L. H.: Microbial reduction of the side-chain carbonyl of daunorubicin and N-acetyldaunorubicin. J. Antibiot. 30: 50-58, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04582-02-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Structure-Activity Correlations in Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Lionel A. Poirier Head, Nutrition & Metabolism Section CMT NCI
OTHER: None

COOPERATING UNITS (if any) Dr. M. Shimkin, University of California, La Jolla
Dr. V. Simmon, Stanford Research Institute
Dr. H. Ruelius, Wyeth Laboratories, Inc., Radnor, Pa.

LAB/BRANCH
Carcinogen Metabolism & Toxicology Branch, Carcinogenesis Program

SECTION
Nutrition & Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mutagenic and carcinogenic activities of previously untested electrophilic agents were investigated. Polymerase A-deficient and WP2(hcr$^-$) E. coli and Salmonella TA 1535 and TA 100 were used in standard plate tests to determine the mutagenic and growth inhibitory activities of the alkyl halides. The ability of alkyl halides to produce lung adenomas in strain A mice was also studied. In general, branching and ease of dissociation increased both the mutagenic and carcinogenic activities of the alkyl halides. However, the addition of polar substituents to alkyl bromides made them too toxic to test for carcinogenic activity, while they retained their mutagenic activities.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The accumulation of evidence has indicated that the activated form of most, if not all, chemical carcinogens consists of a reactive electrophile. The aim of these studies is to determine the extent to which the electrophilic hypothesis of carcinogenesis may be used to predict the potential carcinogenic activities of unkown compounds. Mutagenesis towards microbial systems in vitro is used to obtain a rough measure of a compound's ability to produce reactive intermediates. Guidelines for assessment of the carcinogenic potential of a chemical are developed from a knowledge of its chemical reactivity, metabolic pathways and mutagenic activity.

Methods Employed: The carcinogenic activities of previously untested compounds are tested by standard protocols, generally by the production of mouse lung adenomas in strain A mice. Mutagenicity studies are being performed with the standard and modified Ames assay with Salmonella, with polymerase A-deficient E. coli and with E. coli WP2(hcr$^-$).

Major Findings: The mutagenic and carcinogenic activities of several alkyl halides have been determined. Virtually all tested compounds are mutagenic to one or more of the tester strains employed. In general, branching and ease of ionization, which increase the electrophilic reactivity of these agents, also tended to increase their mutagenic activities. Both factors also seemed to increase the carcinogenic activities of the alkyl halides towards mouse lung. However, polar group substitution into alkyl bromides did not appreciably alter their mutagenic activities, but made them too toxic to be tested in the mouse lung adenoma assay.

The effects of anchimeric assistance on the mutagenic and carcinogenic activities of the alkyl chlorides are under current investigation.

Significance to Biomedical Research and the Program of the Institute: The aim of these studies is to increase the base of theoretical knowledge by which the potential carcinogenic hazards to man of previously untested carcinogens can be estimated. The evidence accumulated to date indicates that among the alkyl halides mutagenic activity more closely parallels electrophilic reactivity than does carcinogenic activity.

Proposed Course of Project: Other classes of electrophilic agents will be tested for their carcinogenic and mutagenic activities. The role of anchimeric assistance in the carcinogenic and mutagenic activites of the alkyl chlorides will be examined.

Publications

Poirier, L.A.: Mutagenicity prescreens for chemical carcinogens. In Montesano, R., Bartsch, H., and Tomatis, L. (Eds.): Screening tests in Chemical Carcinogenesis. IARC Scientific Publication #12, pp. 15-24, Lyon, 1976.

Poirier, L.A. and Simmon, V.F.: Mutagenic-carcinogenic relationships and the role of mutagenic screening tests for carcinogenicity. Clin. Toxicol. 9: 761-771, 1976.

Fluck, E.R., Poirier, L.A. and Ruelius, H.W.: Evaluation of a DNA polymerase-deficient mutant of E. coli for the rapid detection of carcinogens. Chem-Biol. Interactions. 15: 219-231, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04583-02-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Methylation of Ribonucleic Acids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Keiji Nishinaga	Visiting Fellow	CMT NCI
CO-PI:	Richard S. Yamamoto	Research Chemist	CMT NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch

SECTION
Nutrition & Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An acrylamide-agarose composite gel electrophoresis method has been developed to separate the different species of RNA and ribosomes from mouse liver and HeLa cells. The polyribosomes can readily be separated into their components, from monosomes to hexamers. A method to study the type of RNA in the ribosomes has also been successfully developed by using a second dimension electrophoresis. This method is being used to study in vivo binding of ^{14}C-dimethylnitrosamine (DMN) to rRNA. DMN was found to cause disaggregation of polyribosomes.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To compare the methylation of ribosomal RNA (rRNA) by methionine and by dimethylnitrosamine (DMN) in normal and preneoplastic mouse liver.

Methods Employed: Mouse liver RNA is isolated by sucrose density gradient centrifugation and by agarose gel electrophoresis. DMN is chronically administered to mice at standard carcinogenic doses. Double-labelling methods are used to study the differences in the distribution of radio-labelled precursors in the hepatic RNA obtained from normal and from carcinogen-treated mice.

Major Findings: An agarose-acrylamide composite gel electrophoresic method has been developed to separate different species of RNA and ribosomes. A method was developed to study the types of RNA in the polyribosomes. Dimethylnitrosamine (DMN) caused the disruption of polyribosomes, resulting in the production of S-40S subunits.

Significance to Biomedical Research and the Program of the Institute: The study of carcinogen binding to large molecules has been done generally by observing RNA, DNA or proteins as a whole. By separating the components it is hoped that the loci of carcinogen attachment will be identified.

Proposed Course of Project: Further studies into the methylation of 28S rRNA and ribosomes will be done. Long term treatment of C3H mice by DMN will be done to compare the chronic effects of carcinogens on specific ribosome degradation and disaggregation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04617-12-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogen Screening Operations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. K. Weisburger	Chief	CMT NCI
OTHER:	T. Benjamin	Chemist	CMT NCI
	C. A. Brown	Microbiologist	CMT NCI

COOPERATING UNITS (if any)
Dr. M. B. Shimkin, University of California, La Jolla; Dr. B. Ulland, FCRC, Ft. Detrick; Dr. M. Wenk, Microbiological Associates, Inc., Bethesda, Md.; and Dr. A. B. Russfield, Mason Research Institute, Worcester, Mass.

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The chronic effects of environmental chemicals or of mixtures of chemicals are determined in suitable animal models. Factors influencing the outcome such as sex, strain, and species of animal are investigated.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: Responsibilities are assumed on the design, performance and evaluation of studies in either contract or inhouse laboratories, on the chronic effect and carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvements in sensitivity, speed and economy. Short-term assays are under development, taking into account the need to secure biochemical activation of most environmental agents. Guidelines for assessment of the carcinogenic potential of chemicals are developed, by consideration of their chemical structures, metabolic pathways and mutagenic activity.

Methods Employed: Attempts are made to secure information on chemicals representing the greatest hazard to the largest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols as well as newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized. The carcinogenic and mutagenic activities of various compounds are determined in appropriate systems, either directly or after metabolic activation. Exploratory meetings with relevant national and international organizations in industry, in government and in university environments are setting the stage for a concerted development effort in this area.

Major Findings: Industrial or environmental chemicals. In the relatively rapid strain A mouse bioassay system, it was found that certain metallic salts which have been shown to have some carcinogenic effects in long term assays in mice and rats were positive in the strain A mouse test. Some of the contaminants found frequently in water supplies in the U. S. were tested in the same system. Of all the compounds only bromoform gave a positive response in the strain A mouse.

A limited test with o-toluidine in young, female Sprague-Dawley rats showed that despite receiving a very large single dose, mammary tumors were not induced by the treatment.

Significance to Biomedical Research and the Program of the Institute: Many factors in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project: Continuing efforts are required 1) to assess relative priorities of materials to be tested, 2) to undertake studies on variations in the protocols to simplify or to develop novel test techniques which are more sensitive, more specific and more economic, and 3) to apply such tests to identify carcinogenic hazards in the environment.

Publications

Weisburger, E. K.: Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. Cancer (in press).

Ulland, B. M., Page, N. P., Squire, R. A., Weisburger, E. K., and Cypher, R. L.: A carcinogenicity assay of Mirex in Charles River CD rats. J. Natl. Cancer Inst. 58: 133-140, 1977.

Weisburger, E. K.: Structure-activity relationships in carcinogenic drugs. In Bungaard, H., Jull, P. & Kofod, H. (Eds.). Alfred Benzon Symposium X - Drug Design and Adverse Reactions. Denmark, Munksgaard (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04618-12-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Endogenous and Exogenous Factors in Chemical Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. Evarts	Veterinary Medical Officer	CMT NCI
OTHER:	C. A. Brown	Microbiologist	CMT NCI
	T. Benjamin	Chemist	CMT NCI
	E. K. Weisburger	Chief	CMT NCI

COOPERATING UNITS (if any)
Dr. John Baker, University of Mississippi
Dr. Emil H. White, Johns Hopkins University
Dr. C. Michejda, National Science Foundation

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.3	2.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects structural alterations may have on the carcinogenicity or toxicity of various compounds are determined. Methods to inhibit the action of known carcinogens by administration of other compounds are devised. The biochemical, morphological, and physiological bases for such inhibitory effects are studied.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal with the development of systems which lead to the prevention of the processes of chemical carcinogenesis and a study of the mechanisms related thereto.

Methods Employed: Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically tailored structures or properties are given together with chemical carcinogens to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings: (1) Effect of hydroxylamine on spontaneous mammary tumors of C3H mice. Earlier studies from this laboratory showed that hydroxylamine inhibits the spontaneous mammary tumors of C3H mice. When hydroxylamine administration to mice was started at the age of 13 weeks or earlier, it had a marked effect on the morphology of the mammary gland and on the incidence of mammary tumors. However, when hydroxylamine administration was started later in life it either failed to decrease or even increased the incidence of tumors though the same morphological effect on the ovaries persisted. A publication on this study is in press.

(2) Effect of hydroxylamine on 7,12-dimethylbenz(a)anthracene induced mammary tumors of rats. Hydroxylamine had a growth promoting effect on rat mammary gland when administration was started at the age of four weeks contrary to the action in mice. It also shortened the time for the appearance of the first palpable tumors after DMBA administration to female Sprague-Dawley rats at the age of 50 days. When hydroxylamine was given two weeks after DMBA administration, the result was more severe atrophy of the gland than that caused by DMBA by itself. At this time it also reduced the number and size of tumors induced by DMBA and lengthened the median latency period for tumor appearance. These experiments showed the protective effect of hydroxylamine on both spontaneous and carcinogen induced mammary tumors when the administration of this chemical was started at the appropriate time. Further studies are needed to explain the mechanism of this protective effect.

(3) Effect of L-tryptophan on liver carcinogenesis induced by diethylnitrosamine and 3'-methyl-4-dimethylaminoazobenzene. In earlier studies L-tryptophan has been reported both to promote or to reduce the number of liver tumors induced by nitrosamines. However, there were no studies on the effect of tryptophan on azo-dye liver carcinogenesis. A study performed in our laboratory showed that L-tryptophan had a protective effect and reduced the number of anaplastic tumors induced by these carcinogens. Further studies on the mechanism of this effect, including other dietary factors such as pyridoxine and other B vitamins are needed.

(4) Effect of sulfaguanidine on carbon tetrachloride (CCl_4) induced lesions of rat liver. A project was undertaken to determine whether sulfaguanidine would protect rats from the hepatocarcinogenicity of CCl_4, as it protected mice from the acute toxic effects. Various schedules of administration of CCl_4 and sulfaguanidine were followed. The hepatic lesions are now being evaluated by electron microscopy.

(5) Effects of structure on chronic toxicity of nitroso compounds. The studies in this area are continuing. One compound, α-ureidodimethylnitrosamine, synthesized by Dr. C. Michejda, induced stomach adenomatosis in NIH Swiss mice, especially the males. Investigation of this effect in other strains will continue.

Significance to Biomedical Research and the Program of the Institute: The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project: A number of the tests for chronic toxicity and carcinogenicity presently underway or planned to go on stream shortly will require considerable effort for several years. Additional emphasis will be placed on methods for inhibiting the carcinogenic process and on understanding the various steps leading to such inhibitions. Thus studies will include variations in endogenous factors as well as select exogenous materials.

Publications

Evarts, R. P. and Brown, C. A.: Morphology of mammary gland, ovaries and pituitary gland of hydroxylamine fed C3H/HeN mice. Lab. Invest. (in press).

Evarts, R. P.: Effect of L-tryptophan on diethylnitrosamine and 3'-methyl-4-N-dimethylaminoazobenzene hepatocarcinogenesis. Food Cosmet. Toxic. (in press).

Weisburger, E. K.: Cancer-causing chemicals. Chemistry 50: 42-48, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04619-12-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on the Metabolism of Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Grantham	Chemist	CMT NCI
OTHER:	L. C. Mohan	Chemist	CMT NCI
	T. Benjamin	Chemist	CMT NCI
	S. V. Gothoskar	Visiting Associate	CMT NCI
	T. V. Reddy	Visiting Fellow	CMT NCI
	P. P. Roller	Chemist	CMT NCI
	E. K. Weisburger	Chief	CMT NCI

COOPERATING UNITS (if any)
Analytical Chemistry Section, CMT, NCI
Nutrition and Metabolism Section, CMT, NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolic pathways of chemical carcinogens, generally various aromatic amines, are studied both in animals or in isolated tissues. Metabolites are separated by various techniques such as thin-layer, column, gas-liquid, or high pressure liquid chromatography. Identification is made through physico-chemical means such as mass spectrometry, nuclear magnetic resonance and ultraviolet spectra. Interaction of metabolites with cellular constituents is determined. The effect inhibitors or promoters of the carcinogens exhibit on the metabolic patterns is studied.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors and accelerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed: Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide and related compounds in various animal species. This includes study of the enzyme systems concerned with certain metabolic steps. Procedures are developed and applied for the separation, purification and analysis of macromolecular constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals. The metabolism of chemical carcinogens and related compounds, and interaction with host targets are examined in vivo and in vitro in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings: (1) Metabolism of 2,4-toluenediamine (2,4-TDA). Studies on the metabolism of this polyurethane foam intermediate are continuing. By utilization of combinations of high pressure liquid chromatography, gas-liquid chromatography and thin layer chromatography, certain new metabolites such as N-acetoxy-, hydroxylamino- and nitroso-compounds have been identified. These results were confirmed by high resolution mass spectrometry.

(2) Metabolism of 2,4-diaminoanisole. In rats this ^{14}C-labeled hair dye ingredient was excreted to the extent of 81% in 24 hours and 94% by 48 hours, the overwhelming portion being in the urine. About 35% of the urinary activity was due to water-soluble, non-extractable material, 23% as free metabolites, 10% as glucuronide conjugates and 6% as sulfate conjugates. Major urinary metabolites were 2,4-diacetylaminoanisole, and 4-acetylamino-2-aminoanisole. There was also the demethylated compound, 2,4-diacetylaminophenol, in both the free and glucuronide fractions. Three additional compounds with M/e of 238 are under investigation. Indications are one is a ring-hydroxy compound, one is a possible N-hydroxy derivative, while the structure of the third metabolite has not been classified. In vitro studies of the metabolism of diaminoanisole are being conducted to determine which cellular fraction is responsible for metabolic conversion. The effects of other variables such as enzyme induction and environmental factors are being investigated. In rat liver cytosol 4-acetamido-2-aminoanisole was the major product from in vitro acetylation of the diaminoanisole.

(3) Metabolism of 2-aminoanthraquinone (2-AAQ) in rats. In addition to the previously reported metabolites, it appears that a new type of metabolite, 2-N-formamidoanthraquinone, is a urinary metabolite of 2-AAQ. This reaction may be more general than previously assumed.

Significance to Biomedical Research and the Program of the Institute: By the utilization of certain chemical carcinogens, the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps from the primary interaction between an agent and cellular constituents, followed by multiplication of abnormal cells which in turn can undergo further transformations, there are a number of points where one could prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to comprehend fully the sequence of complex reactions leading to cancer and eventually to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project: The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Publications

Gothoskar, S. V., Benjamin, T., Roller, P. P. and Weisburger, E. K.: Metabolic fate of 2-aminoanthraquinone, a probable hepatocarcinogen and a nephrotoxic agent in the Fischer rat. Proceedings of the Third International Symposium on the Detection and Prevention of Cancer. New York, Marcel Dekker, Inc. (in press).

Grantham, P. H., Giao, Ng. Ba., Mohan, L. C., Benjamin, T., Roller, P. P. and Weisburger, E. K.: The metabolism of 6-aminochrysene in the rat. Eur. J. Cancer 12: 227-235, 1976.

Grantham, P. H., Mohan, L. C. and Weisburger, E. K.: Metabolism of N-2-fluorenylacetamide in X/Gf strain mice: lack of a correlation between biochemical interaction and carcinogenicity. J. Natl. Cancer Inst. 56: 649-651, 1976.

Mohan, L. C., Grantham, P. H., Weisburger, E. K., Weisburger, J. H. and Idoine, J. B.: Mechanisms of the inhibitory action of p-hydroxyacetanilide on carcinogenesis by N-2-fluorenylacetamide or N-hydroxy-N-2-fluorenylacetamide. J. Natl. Cancer Inst. 56: 763-768, 1976.

Poirier, L. A., Grantham, P. H. and Rodgers, A. E.: The effects of a marginally lipotrope-deficient diet on the hepatic levels of S-adenosylmethionine and on the urinary metabolites of 2-acetylaminofluorene in rats. Cancer Res. 37: 744-748, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04620-12-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Mode of Action of Chemical Carcinogens - Chemical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Benjamin	Chemist	CMT NCI
OTHER:	E. K. Weisburger	Chief	CMT NCI

COOPERATING UNITS (if any)
Analytical Chemistry Section, CMT, NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project serves as a supporting function to the studies on metabolism of various toxic or carcinogenic agents in this Branch. The purity of compounds being investigated for toxicity or carcinogenicity is determined along with pertinent physical characteristics, useful in the design of the experiments. Possible metabolites of various aromatic amines, such as 2,4-toluenediamine, 2,4-diaminoanisole, and 2-aminoanthraquinone are synthesized as reference materials for metabolism studies. Methods for separation of aromatic amines and their metabolites are investigated.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens. Model studies on the reactions of chemical carcinogens with potential targets are performed.

Methods Employed: Properties of chemicals acquired or synthesized for the small scale bioassay of chemical carcinogens are investigated to determine the purity of these materials. In model experiments the reactions of certain of these agents, particularly their primary and ultimate reactive forms, with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings: Metabolites of aromatic amines. The following new compounds have been synthesized as reference materials for metabolic studies: 3-hydroxy-2-aminoanthraquinone, 2-formamidoanthraquinone, 1- and 6-hydroxy-2-acetamidoanthraquinone, 2-acetamido-4-aminoanisole, 2,4-di(acetamido)-anisole, 2-amino-4-acetamidoanisole, 2-hydroxylamino-4-aminoanisole, 2-(N-hydroxyacetamido)-4-acetamidoanisole, 2-(N-acetoxyacetamido)-4-acetamidoanisole, 2-formamido-4-acetamidoanisole, 2,4-di(acetamido)phenol, 2,4-di(acetamido)acetoxyphenol, 2-amino-4-nitrosotoluene, 2-acetamido-4-nitrosotoluene, 2-amino-4-hydroxylaminotoluene, 2-acetamido-4-hydroxyl-aminotoluene, and 2-acetamido-4-(N-acetoxy)-acetamidotoluene.

Significance to Biomedical Research and the Program of the Institute: The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Branch.

Proposed Course of Project: This project will continue along the same lines as previously.

Publications

Weisburger, E. K., Grantham, P. H. and Benjamin, T.: Separation and analysis of various carcinogenic aromatic amines and their metabolites. Proceedings of the Third International Symposium on the Detection and Prevention of Cancer. New York, Marcel Dekker, Inc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04680-07-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development and Application of In Vitro Systems Involving Epithelial Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. J. Wilson	Chemist	CMT NCI
OTHER:	E. K. Weisburger	Chief	CMT NCI
	J. Elliott	Microbiologist	CMT NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION
Nutrition and Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Epithelial-like cells derived from livers of infant Fischer rats are maintained in culture, either continuous or after frozen storage. Ultrastructural alterations produced by long term culture and frozen storage are being examined. Transformation of the cells to a malignant form by various chemical carcinogens is being studied.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: Successful culture of epithelial-like cells derived from livers of 8 to 10 day old Fischer strain 344 rats and malignant transformation of such cultured cells by a variety of chemical carcinogens were discussed in previous reports. Since neoplasia in man and animals involves mainly epithelial cells, our principal aim in current investigations has been to further determine the potential of in vitro epithelial systems for use in rapid bioassay procedures for chemical carcinogens and in examining the biochemical mechanism of carcinogenesis.

Two problems encountered in past investigations were the relatively long time required to develop cultures that were proliferating adequately to provide enough cells for biochemical investigations and the fact that some sublines of control cells underwent spontaneous malignant transformation as demonstrated by tumor formation following injection of cells into syngeneic hosts. Unfortunately, no distinctive morphological changes were apparent in the cultured cells that induced tumors. In addition, the spontaneous transformation was not correlated with a specific time in culture nor with the number of times a line had undergone subculturing. Therefore, we have emphasized the development of methods for producing a greater number of cultures with similar characteristics so that studies can be repeated as required in more replicable systems. Investigation of pertinent biochemical properties of untreated or chemically exposed cultured cells in respect to the effects of various cultural conditions and in comparison to the characteristics of liver tissue from whole animals should help assess the value of this in vitro system.

Methods Employed: Methods for selecting rat liver cells for culture continue to be developed in this laboratory. This involves detaching and transferring islands of epithelial cells, both mechanically and by trypsinization from cloning cylinders, so that many homogeneous sublines can be cultured from one primary culture.

Previous studies in this laboratory have shown that frozen storage of cells did not alter enzyme levels or cause transformation. To further investigate the effects of frozen storage as well as long term continuous culture on the ultrastructure of rat liver cells, electron microscopy studies have been initiated.

To investigate the potential carcinogenicity of 2-aminoanthraquinone, rat liver epithelial cells were exposed to AAQ for various time periods and at several concentrations.

Major Findings: (1) Chemical transformation of rat liver cells with 2-aminoanthraquinone. Preliminary results indicate transformation of rat liver epithelial cells exposed to AAQ for three months.

Significance to Biomedical Research and the Program of the Institute: An assay system which can be designed to give results more rapidly, specifically, and reliably than the customary long-term tests for carcinogenicity is highly desirable with the dual purpose of studying fundamental phenomena and the detection of harmful agents in our environment. Full exploitation of workable in vitro systems has not been achieved. It is our goal to develop such systems; an additional advantage of the in vitro systems is that eventually they can be established utilizing cells from man. Hence carcinogenesis in vitro with human cells avoids the question of species difference, so often raised in evaluating the significance of carcinogen bioassay systems.

Proposed Course of Project: These epithelial cells will be carefully examined for suitability as a carcinogen screening system. The relevant morphological properties in both normal and carcinogen treated cells will be investigated thoroughly. Extensive efforts will deal with the biochemical properties of both the normal and the transformed cells in culture. Basic biochemical mechanisms of the system will be investigated.

Publications

Idoine, J. B., Elliott, J. M., Wilson, M. J. and Weisburger, E. K.: Rat liver cells in culture: effect of storage, long-term culture, and transformation on some enzyme levels. In Vitro 12: 541-543, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04688-07-CMT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Investigations on Systems Leading to Cancer in the Colon and Small Intestine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. K. Weisburger	Chief	CMT NCI
OTHER:	C. A. Brown	Microbiologist	CMT NCI
	R. P. Evarts	Veterinary Medical Officer	CMT NCI
	T. Benjamin	Chemist	CMT NCI

COOPERATING UNITS (if any)
Dr. M. L. Wenk, Microbiological Associates, Inc.

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Animals are injected with chemical carcinogens known to induce tumors of the colon and small intestine. Alteration of these tumorigenic effects by various dietary constituents such as proteins, fats, carbohydrates, antioxidants, or bulk material is investigated. Characterization of some of the enzymes as well as their levels in the induced tumors is performed.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: In the United States and Western Europe the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in women, after breast and uterus. However, in other countries such as Japan and Africa, colon cancer incidence is much lower, thus suggesting that environmental factors are associated with the etiology of this important disease. Within recent years several new agents were discovered by which colon cancer could be induced in animals. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon in animals thereby gaining insight into conditions possibly affecting humans.

Methods Employed: Rats, mice and other rodent species were treated with chemicals known to lead to colon cancer, such as 1,2-dimethylhydrazine and azoxymethane. The animals are placed on diets mimicking those utilized in various parts of the world with regard to quantity and quality of protein, fat, carbohydrates, micronutrients, and the amount of bulk material such as cellulose in the diet. After appropriate periods of time the animals are carefully autopsied and the lesions are examined microscopically.

Major Findings: (1) β-Glucuronidase and cytochrome oxidase in colon neoplasms from rats injected with azoxymethane. Rats are injected once weekly for 10 weeks with a dose of azoxymethane which leads to many tumors, well distributed throughout the colon. Rats are killed at 4, 6 and 8 months after the first injection. It was found that all the types of neoplasms induced had an elevated β-glucuronidase activity. Despite extensive histological and histochemical study it was decided that β-glucuronidase could not be used to differentiate between preneoplastic and neoplastic lesions.

(2) Effect of BHT on colon tumor induction by azoxymethane. BHT, used in many foods as an antioxidant, has inhibited the growth of liver or pulmonary tumors in rats or mice given the appropriate carcinogens. A similar type study was initiated on BHT in rats injected with azoxymethane. It was found that the incidence of intestinal tumors was inhibited significantly when the rats received BHT simultaneously with the azoxymethane. If the BHT was given after the azoxymethane, the tumor incidence was not affected. A publication on this subject is in press.

Significance to Biomedical Research and the Program of the Institute: Establishment of a colon cancer model with similarities to the human disease was necessary to study the effects of diet on colon cancer induction since dietary factors are thought to be responsible for the occurrence of human

colon cancer. Our studies suggest that under our experimental conditions, diets containing nutrients thought to be important for the high incidence of human colon cancer in some parts of the world did not increase the incidence of chemically-induced cancer. The limitations of this model are obvious.

Proposed Course of Project: As facilities are available further studies on the dietary effects of colon cancer incidence in experimental animals will be initiated.

Publications

Weisburger, E. K., Evarts, R. P. and Wenk, M. L.: Inhibitory effect of butylated hydroxytoluene (BHT) on intestinal carcinogenesis in rats by azoxymethane. Food Cosmet. Toxicol. (in press).

SUMMARY REPORT

CHEMISTRY BRANCH

July 1, 1976 through September 30, 1977

"Plans, develops, and conducts a research program designed to (1) clarify the molecular biology of carcinogenesis; (2) elucidate the fundamental nature of the interactions of carcinogenic agents, especially chemical, with biological systems in the induction of cancer; (3) define those environmental and endogenous factors which relate to and modify the carcinogenic process; and (4) clarify the metabolic regulatory processes which are related to carcinogenesis."

The goal of the Chemistry Branch is to understand the molecular basis of carcinogenesis with the view toward identifying susceptible populations and preventing human cancer. The research program is designed to understand the molecular basis by which carcinogenic agents cause malignant transformation and identify and characterize those exogenous and endogenous factors involved in carcinogenesis. The Branch seeks to clarify the metabolic interaction of exogenous and endogenous agents in the living organism at the molecular, cellular and organism levels and seeks to understand the consequences of these interactions in terms of cell regulation and carcinogenesis. These processes are studied in biological preparations and cells from experimental animals and humans.

Cancer is a disease in which the genetic information and/or expression is aletered. This altered phenotypic expression may be due to genetic or epigenetic events induced by either xenobiotic or endogenous factors which result in altered patterns of gene control. The central aim of the Branch is to understand how exogenous carcinogens and endogenous factors are processed by enzymatic mechanisms, how the carcinogen is converted to active forms and the nature of the interaction between the latter and the gene action system. Systems involved in the repair of genetic damage induced by carcinogens are studied. Understanding is sought on how the interaction with carcinogens results in the modifications which characterize malignant transformation. The aim of these studies is modification, elimination or prophylaxis of the carcinogenic response in the human population.

Senior staff of the Branch is also responsible for the scientific direction of the Chemistry and Molecular Carcinogenesis Segment.

Cell Growth Regulation Section - *"Studies (1) the biochemical factors controlling cell growth and differentiation and the nature of the mechanisms governing the phenotypic expression of genetic information; (2) chemical, physical, and biological agents capable of inducing alterations in growth behavior and differentiation."* The primary goal of this Section is to define the molecular events of malignant transformation induced by chemical carcinogens, and in particular the nature of the molecules responsible for converting a normal cell to its malignant form.

Three major areas were investigated in this Section.

One of these is the development and improvement of the systems for assay of potent carcinogens using cultured cells. The combination of psoralen and long wavelength ultraviolet radiation has been found to be mutagenic in bacteria and carcinogenic in mice and to induce a decrease in DNA synthesis in circulating leucocyte from patients treated. This combination is being used experimentally for treatment of psoriasis and the cutaneous lymphoma mycosis fungoides in humans. An in vitro assay system has been developed for the measurement of effects of this treatment combination on cell proliferation and DNA synthesis using rapidly dividing human lymphoblastoid cells.

The development of a transformation system using cultured human cells may provide a means to assay potential carcinogens directly on human cells, thus removing the necessity of extrapolating data from other animal cells. This system would also provide important information on the relationship of the response to carcinogenic effect of chemicals between different species and between in vivo and in vitro results. Our previous findings of malignant transformation of human diploid fibroblasts by 4-nitroquinoline-1-oxide and N-methyl-N'-nitro-N -nitrosoguanidine have been confirmed. The properties of the transformed cells were further characterized, including their transplantability into nude mice. A strict requirement for cell division after carcinogen-treatment was a requirement for the development of the transformation.

A quantitative system for transformation using a subclone derived from BALB/3T3, has been improved by selecting a clone more stable, and with higher sensitivity to chemical transformation. This modified system seems to be very useful for routine assay of potent carcinogens.

The second major area of study is the mechanism of chemical carcinogenesis. This is being done by attempts to define genetic and physiological factors affecting carcinogenesis. It includes a study of the relation of these factors to exogenous factors in the incidence of human cancer. One approach is to study cells obtained from patients with cancer-prone genetic diseases, in particular xeroderma pigmentosum (XP), a disease with UV-sensitivity, and ataxia telangiectasia (AT), a disease with x-ray sensitivity. Cell fusion studies have demonstrated at least five different DNA repair defects among XP fibroblast strains. A detailed comparison of the clinical features of these diseases was made. XP cells were found to be extremely sensitive to the cytotoxic effects of the potent carcinogens, 4NQO and benzo(a)pyrene-diol-epoxide I. The higher sensitivity was explained by a lack of excision of the carcinogen-DNA adducts in the XP cells.

Another approach to define the genetic factors affecting chemical carcinogenesis is to clarify the nature of cell mutants of different sensitivities to chemical carcinogens. Cell mutants isolated from a BALB/3T3 clone were classified into four different categories according to their sensitivities to chemical carcinogens. They exhibited parallel sensitivities to transformation by various physical and chemical carcinogens tested, suggesting that these mutants may be very useful for studying the genetic and biochemical factors involved in the process of cell transformation by carcinogens.

Studies on the physiological factors affecting transformation has supported a hypothesis that transformation by chemicals is induced through error-prone-DNA repair process, because the first S-phase after chemical-treatment is a rigorous requirement for the fixation of the transformation and this process is inhibited by caffeine. Biochemical studies on the 4NQO-adducts to DNA bases under various conditions support the above hypothesis.

A physiologically active molecule primarily responsible for malignancy is being examined. Temperature-sensitive and cold-sensitive cell mutants affected in the expression of transformed phenotype were isolated and characterized. Insertion of double drug resistance into these mutants and optimum conditions for cell hybridization have been established in order to investigate the genetic factors controlling the expression of transformed phenotypes.

Molecular Carcinogenesis Section - *"Studies (1) the metabolic effects on tissues and cells resulting from their exposure of chemical carcinogens and the relationship between these effects and malignant transformation; (2) the metabolic activation and detoxification of the polycyclic hydrocarbons and the relationship of this metabolism of individual susceptibility."*
This section studies the molecular events of malignant transformation induced by chemical carcinogens, in particular those of the polycyclic hydrocarbon class. The aim is to understand the enzymatic conversion of carcinogens to either detoxification forms or to the active carcinogenic metabolite. During evolution humans have been exposed to foreign chemical compounds (including carcinogens) and has developed metabolic systems for their detoxification and elimination. These systems primarily involve microsomal cytochrome P-450 mixed-function oxygenases, but also include epoxide hydratases and conjugating enzymes. The vast majority of foreign compounds are processed by these enzyme systems. The mixed-function oxygenases are influenced by a variety of environmental factors such as drugs, pesticides or carcinogens, and are influenced by the nutritional and hormonal state of the animal. The age, sex and genetic makeup also determines enzyme activity. Work in this laboratory provided the key studies which showed this enzyme system to be responsible for the activation of procarcinogens to what may be their ultimate carcinogenic form.

A primary goal is to define the enzymatic mechanism by which polycyclic hydrocarbons are either activated to carcinogenic forms or to detoxified products. As these enzymes are characterized and sensitive methods are developed for their assay, it may be possible to characterize an individual's enzymatic makeup with respect to carcinogen metabolism and to understand the relationship between this metabolism and individual susceptibility to polycyclic hydrocarbon carcinogenesis. The known sequence of PCH metabolism is as follows: The first step is oxygenation by the microsomal mixed-function oxygenases, one indicator of which is aryl hydrocarbon hydroxylase (AHH). Very sensitive methods have been developed for the latter (see below) which are applicable to human tissues. Secondly, epoxides are hydrated to dihydrodiols by oxide hydratase. The oxides are also metabolized by glutathione S-transferases. The phenols and dihydrodiols are conjugated by UDP glucuronic acid. Sensitive assays (see below) for each of these systems as well as a method for total metabolite analyses by high-pressure liquid chromatography (HPLC) have been developed.

Aryl hydrocarbon hydroxylase (AHH), an enzyme system metabolizing benzo(a)pyrene, has been analyzed in human monocytes and lymphocytes. It was found that in respect to the induced level of enzyme, there was no correlation between monocyte and lymphocyte activity. Thus, the activity in each of these different cell types may reflect different forms of the mixed-function oxidase.

A study has been completed on the genetic parameters governing AHH induction. Over 40 sets of identical and non-identical twins were studied on 2 to 3 separate occasions to determine the relationship between AHH activity in identical and non-identical twins. It was found that both individuals in a set of identical twins had very similar AHH activity. The similarity was also observed, but to a lesser extent, in fraternal twins. This suggests that a relatively small number of genes regulate AHH activity in human monocytes.

Highly purified forms of cytochrome P-450 were found to exhibit substrate selectivity and product regio-selectivity and stereo-selectivity in respect to benzo(a)pyrene and 7,8 diol BP metabolites. Thus of six different forms of the enzymes examined each is unique and the different forms of enzyme may relate to the regulation and detoxification pathways. This study may relate to the mechanism of individual variation to carcinogen susceptibility and is an important step forward in understanding the molecular basis for carcinogen activation.

A combination of gravity-flow and high-pressure liquid chromatography has been developed to separate all the primary BP metabolites. A high-pressure liquid chromatographic system has been developed to analyze secondary metabolites of BP. The detailed mechanism of the enzymatic formation of a highly mutagenic metabolite, r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene has been elucidated which may be the ultimate carcinogenic form of benzo(a)-pyrene. This diol-epoxide is also the most potent carcinogen among BP derivatives in experimental animals.

The activity of the mixed-function oxygenase, aryl hydrocarbon hydroxylase, was assigned to a human chromosome number two by comparing the relationship to the expression of human isozymes in somatic mouse-man cell hybrids. The results suggested that the gene(s) regulating the rate of synthesis of the oxygenase is in close proximity to the structural gene and that both activities are on the same chromosome.

A study demonstrated that the phenol, diol and quinone metabolites of BP are all conjugated to the sulfate and become water-soluble products. This may represent a key detoxification path for oxidized polycyclic hydrocarbon metabolites.

Nucleic Acids Section - "*Studies (1) interaction of chemical carcinogens and their analogs with nucleic acids and with enzymes regulating nucleic acid metabolism; (2) the effect of these agents on the subcellular organization of nucleic acid macromolecules.*" Several studies of the chemical and biological effects of the benzo(a)pyrene metabolite, 7,8 diol 9,10 epoxide, were conducted during the past year. One study with both the DNA from the plasmid Col E1 of E. coli and from SV40 (both circular molecules) showed specific conformational

changes on reaction with the carcinogen. These involved relaxation and linearization of the molecule. Most of the carcinogen was bound to guanosine residues, but a small fraction was bound to adenine. The binding of adenine caused a local denaturation of the DNA chain which made it susceptible to the action of a single-stranded nuclease. This type of binding, hitherto unreported, may be of a special significance. The cleavage of the circular to linear form suggests that the epoxide may attack the phosphorus diester bond directly in addition to modifying the DNA by binding to the bases. By the use of the BND-cellulose technique and the adenovirus host cell reactivation assay it was found that treatment of cells or viruses with the diol-epoxide caused extensive damage to the DNA which was repaired by an excision-repair mechanism. The ability of normal cells and of cells deficient in excision repair to repair DNA damaged by the diol-epoxide closely parallel their ability to repair DNA inactivated by ultraviolet radiation, suggesting that from a cellular point of view these two agents produce similar DNA damage.

Patients with Xeroderma pigmentosum (XP) and Ataxis telangiectasia (AT) have a greater cancer incidence than the general population. Repair of DNA damage by a variety of chemical agents was measured in several different cell lines from such patients. After treatment with N-methyl-N'-nitro-N-nitroguanidine (MNNG), AT cells showed 2 to 5 times less repair synthesis than did normal cell lines. This finding is significant since previously published observations on the AT repair responses showed only sensitivity to x-rays. It is likely that MNNG induces repair at the growing point regions of the DNA and it will be important to determine whether AT cells exhibit further abnormalities in this phase of DNA synthesis.

Studies were made of an interesting mutant of adenovirus 2 which grows on 8 strains of human tumor cells but that does not make plaques on normal human fibroblasts. Such a study might reveal important differences between normal and tumor cells in control of DNA replication. DNA synthesis by the mutant virus is slower than that by the wild-type virus infecting normal human fibroblasts.

A beginning has been made into the study of possible mechanisms whereby heavy metals may act as mutagens or carcinogens. Specific proteins which covalently bind these metals are required for the transport into the cell. The cellular level of Cd-binding protein is dependent upon calcium uptake and many other factors. Transformation of chick embryo cells by Rous sarcoma virus enhanced the level significantly.

The significance of several isoacceptor tRNAs and multiple codon responses for specific amino acids is under continual study. A recently completed very extensive survey of isoacceptor tRNAs and their codon responses is the basis for an attempt to relate the concentrations of each tRNA and the frequency of usage of the corresponding codons in mRNA. The extent to which errors and possible mutations may occur by misrecognition of tRNAs by synthetases has been partially evaluated by the use of mammalian enzymes and tRNA from E. coli. Only a low level of misrecognition in a few cases was found, but a search for specific misrecognition will continue.

Protein Section - "*Studies (1) the relationship of proteins to the carcinogenic process; (2) interactions between carcinogenic agents and proteins; (3) changes in proteins occuring as a result of or concomitant with the administration of carcinogenic agents; relates the genetic control of such proteins to inheritance in laboratory animals and man.*" Based on findings made in other laboratories, it has been hypothesized that the level of certain serum glycoproteins, especially orosomucoid and haptoglobin, are controlled by events at the plasma membrane of the liver cell. Specifically being tested is the idea that adsorption of desialylated glycoproteins to specific receptor sites on the membrane serve as signals for stimulation of synthesis of the specific protein recognized. Injection into rabbits of purified haptoglobin and haptoglobin from which the sialic acid residues had been removed was followed by increases in the level of serum haptoglobin which were approximately the same for both forms of injected protein. Because this result was difficult to interpret by any molecular hypothesis, steps are being taken to repeat similar experiments using liver slices and primary hepatocyte cultures.

Electrophoretic analyses of microsomal proteins have been performed in order to define changes in specific proteins which give rise to increases in aryl hydrocarbon hydroxylase activity. The changes in electrophoretic patterns which were observed after treatment with the carcinogen 3-methylcholanthrene were compared with those found after phenobarbital treatment. With the carcinogen, two microsomal proteins were induced in several strains of rat and there was evidence suggesting that a third microsomal protein was decreased in amount. Similar changes were observed after phenobarbital treatment, but the responding proteins were of lower molecular weight than those observed after carcinogen treatment. In addition to understanding the mechanisms that control synthesis of these proteins, it is hoped that these techniques will be useful in identifying genetic variants which may exist in the human population.

Previous work in this Section has led to methods for identification of phosphoproteins and sialoglycoproteins in milk as well as in mammary tissue. These studies have been extended to permit the identification of these proteins and also hyaluronic acid in connective tissue, secretions within cells, and the alveolar lumina in ducts in mammary tissue. It is likely that such methods will be valuable in the histological examination of mammary lesions that are difficult to classify by morphology alone.

Caseins from mouse milk were further characterized electrophoretically and could be identified among other breast proteins in mice at mid-pregnancy. The production of caseins by explants of mouse mammary tissue was dependent on the level of prolactin in the medium. The measurement of casein produced following a 5-day incubation was used as a bioassay for prolactin.

Estrone and several related steroids and metabolic products therefrom have been evaluated for mutagenic activity using the Ames test. The number of revertants observed was no more than twice control levels and the statistical significance of the result remains to be determined. Because such substances are continuously present in mammary and similar tissues, even very low mutagenic action might be important. Further studies are being undertaken to assess the significance of these findings.

The heterogeneous nuclear RNA is the primary transcription of the gene and in addition to specific messengers for specific proteins contains all RNA that might be involved in the control of genetic expression. Substantial progress has been made in the electrophoretic characterization of this diverse group of molecules. The aggregates of hnRNA may be dissociated by use of dimethylsulfoxide and enzymatic removal of DNA. The applicability of ultracentrifugal and electrophoretic analysis in estimating the chain lengths of these molecules is under study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04475-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Factors Controlling Hepatic Synthesis of Acute-Phase Plasma Glycoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Hooper	Research Associate	CH NCI
OTHER:	A. C. Peacock	Head, Protein Section	CH NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of experimental inflammation and binding of purified desialylated haptoglobin to hepatocytes on plasma haptoglobin levels were studied in the rabbit in vivo. Synthesis of haptoglobin and other plasma acute-phase proteins was further investigated in rat liver slices in vitro and will be extended to primary hepatocyte cultures. In these in vitro systems, factors under investigation as possible mediators of the acute-phase plasma glycoprotein response to inflammation include glucocorticoids, insulin, glucagon, asialohaptoglobin, complement, leucocyte endogenous mediator, and endotoxin.

In continuation of a previous project, the subunit composition of isolated haptoglobin 2-1 polymers will be studied by ultracentrifugation methods and electrophoresis for comparative molecular weight determinations.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine the factors acting on the hepatocyte membrane that signal increased plasma acute-phase glycoprotein synthesis in response to inflammation.

Methods Employed: 1) Quantitative polyacrylamide gel electrophoresis, 2) liver slice incubation, and 3) primary hepatocyte culture.

Major Findings: Experimental inflammation (turpentine-induced sterile abscess) evokes increased plasma haptoglobin levels in vivo and stimulates protein synthesis in subsequently prepared rat liver slices. Rabbit plasma haptoglobin levels are not differentially affected by injection of homologous asialohaptoglobin (which binds to hepatocyte membranes) compared to "native" haptoglobin.

In vitro paired liver slices taken from normal rats incorporated labelled amino acids or D-glucosamine into protein approximately linearly for up to twelve hours. Cortisol, insulin, and glucagon were without consistent effect in physiologic levels in this system. Endotoxin and increasing concentrations of glucocorticoids in vitro depressed the rates of amino acid incorporation.

Significance to Biomedical Research and the Program of the Institute: A major aspect of the systemic response to inflammation is a striking increase in the hepatic synthesis of several plasma glycoproteins with little change in albumin synthesis. Although glucocorticoids have been shown to have a permissive effect in vivo, and crude preparations from stimulated leucocytes have accelerated synthesis of plasma glycoproteins in vivo and in vitro, the specific factor or factors mediating this response are uncertain. In progress is development of an in vitro system for hepatic protein synthesis. The system should have intact surface membrane receptors and be sufficiently viable to respond to purified materials over the several hour period known to be required for stimulation of acute-phase glycoprotein synthesis in vivo. Particular emphasis is directed toward the hypothesis that the binding of asialoglycoproteins to their hepatic membrane receptors (as elucidated by Ashwell and co-workers) serves as a feedback signal for additional glycoprotein synthesis. The release of leucocyte lysosomal neuraminidase at the site of inflammation is hypothesized to result in an increased fraction of circulating glycoprotein in the desialylated form.

The phenomena under investigation are common to inflammatory states induced by infectious diseases and cancer. Elevated levels of plasma glycoproteins are of uncertain value to the host, although the ability to bind released proteolytic enzymes or free hemoglobin released by hemolysis may be of protective value in infectious diseases, leukemia, and other neoplastic conditions. Study of cell surface receptors and recognition phenomena, such as those involving sialic acid and other carbohydrate moieties, are also important in understanding regulation of cell growth and replication.

Proposed Course of Project: Extension of this project will include investigation of possible mediators other than asialoglycoproteins (leucocyte endogenous mediator, complement) and study of intracellular events effecting mediator-induced increases in protein synthesis.

Publications

Hooper, D. C. and Peacock, A. C.: Determination of subunit composition of haptoglobin 2-1 polymers using quantitative gel electrophoresis. Fed. Proc. 35: 1446 (abstract no. 475), 1976.

Hooper, D. C. and Peacock, A. C.: Determination of the subunit composition of haptoglobin 2-1 polymers using quantitative densitometry of polyacrylamide gels. J. Biol. Chem. 251: 5845-5851, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04476-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Detoxification Processes of Benzo(a)pyrene (BP)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Nemoto	Visiting Fellow	Cancer Institute Tokyo, Japan
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Molecular Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The enzymes, which produce water-soluble materials by conjugating with glutathione, glucuronic acid and sulfate, were investigated for their activities with various kinds of BP metabolites and derivatives. The processes have implications in the excretion of chemical carcinogens from the body.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04476-01-CH

Project Description

Objectives: To understand the metabolic path of carcinogen detoxification.

Methods Employed: Enzymology

Major Findings: Glutathione, glucuronic acid and sulfate transferases are routes of detoxification for chemical carcinogen.

Significance to Biomedical Research and the Program of the Institute: Better understanding of the processing of polycyclic hydrocarbon carcinogens.

Proposed Course of the Project: None

Publications

Nemoto, N. and Gelboin, H. V.: Enzymatic conjugation of benzo(a)pyrene oxides, phenols and dihydrodiols with UDP-Glucuronic acid. Biochem. Pharmacol. 25: 1221-1226, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04477-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on the Control of Benzo(a)pyrene Metabolism in Cells From Animal and Human Sources

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Okano	Staff Fellow	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI
	H. Miller	Chemist	CH NCI
	R. Robinson	Biologist	CH NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Molecular Carcinogenesis

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.1	1.8

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) Blood monocytes and lymphocytes, a readily available source of human tissue, are used to assess in vitro an individual's ability to metabolize benzo(a)pyrene, a model compound of the class of polycyclic aromatic hydrocarbon carcinogenens. (2) Rat liver cells are used to obtain a correlation between the relative AHH activity and inducibility and the pattern of benzo(a)pyrene metabolites as seen by high pressure liquid chromatography (HPLC).

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To observe and to quantitate the metabolic products formed by the oxidation of benzo(a)pyrene in vitro in human peripheral blood monocytes and lymphocytes and in cells from rat or mouse tissues. (2) To determine whether quantitative and/or qualitative differences in benzo(a)pyrene metabolism by selected cells can be correlated to their relative aryl hydrocarbon hydroxylase (AHH) activity and inducibility.

Methods Employed: Lymphocytes and monocytes are separated from other components of fresh human blood by density gradient centrifugation on Ficoll-Hypaque. Separation of monocytes from lymphocytes is by the differential attachment of monocytes to plastic cell culture dishes. Monocytes are cultured for about 40 hours total and lymphocytes are cultured in the presence of 1% phytohemagglutinin and 1% pokeweed mitogen for a total of 64 hours. The final 24 hours before cell harvest in each case are in the presence of 2 ug/ml benz(a)anthracene (BA) to induce AHH activity. (2) Rat liver cell cultures were obtained from sources at NIH and cloned by conventional, published techniques. Clones and the parent cell cultures are induced by 1 ug/ml BA and characterized as to their relative AHH activity and inducibility. (3) After incubation of cells in the presence of benzo(a)pyrene (BP), the relative AHH activity and inducibility of cells is determined by a fluorescence assay using 3-hydroxy-BP as a standard. (4) Cells are also incubated in the presence of radiolabeled BP and BP-(-)trans 7,8-dihydrodiol. Metabolites are extracted from the reaction mixture with ethyl acetate and analyzed by high pressure liquid chromatography (HPLC).

Major Findings: (1) The AHH activity and inducibility of cultured monocytes and lymphocytes from the same individual have been compared. The correlation obtained to date has been poor, but the data is expected to improve due to alterations in the AHH assay which have been made within the last 6 months. As measured by HPLC, both monocytes and lymphocytes can oxidize BP to products which include: 9,10-, 4,5- and 7,8- dihydrodiol, 3-, 9- and possibly 7- phenol, 1,6- and 3,6- quinone and 2 unknowns. The relative ratios of BP metabolites formed by monocytes differs from the ratios of metabolites formed by lymphocytes. Both monocytes and lymphocytes are capable of oxidizing BP-7,8-dihydrodiol further to the highly mutagenic and carcinogenic BP-7,8-dihydrodiol-9,10-epoxide. (2) Several clones of rat liver cells have been isolated. The relative AHH activity and inducibility is being determined for each clone and the parent cell line. A range of AHH activities, and inducibilities, from low to high, has been initially observed. The liver cells examined by HPLC show differences in their ability to metabolize BP, cells from the Buffalo rat forming little or no detectable dihydrodiols.

Significance to Biomedical Research and the Program of the Institute: Peripheral blood mononuclear leukocytes, a readily available source of human tissue, have the ability to metabolize polycyclic aromatic hydrocarbon carcinogens. These cells use the same microsomal mixed function oxygenase system, aryl hydrocarbon hydroxylase, which is found in other tissues. Analysis of benzo(a)pyrene metabolism by the AHH assay and by HPLC will yield quantitative and qualitative data which will hopefully be correlated to carcinogenic potential. The evential goal is the development of in vitro systems which can assess an individual's relative susceptability to polycyclic aromatic hydrocarbon carcinogenesis.

Proposed Course of the Project: Blood monocytes and lymphocytes from various individuals who have different relative AHH activities and inducibilities will continue to be assayed for BP metabolism by HPLC. Animal cell systems which can be used to assess the correlation between AHH activity and susceptibility to carcinogenesis will continue to be developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04478-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Modification of DNA by Chemical Carcinogens and Mutagens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakefuda	Medical Officer	CH NCI
OTHER:	H. Yamamoto	Visiting Fellow	CH NCI
	T. M. Bak	Microbiologist	CH NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(A) The topic of the present study is the structural modification of DNA double helix caused by binding of carcinogens and mutagens. It was found that there were three different types of in vitro binding of benzo(a)pyrene (BaP) metabolite, 7,8 diol 9,10 epoxide BaP, to superhelical circular DNA of plasmid Col E1 and SV40 virus: (1) The covalent binding which does not cause severe steric hindrance on the DNA double helix. The binding with guanine belongs in this category. (2) The binding which causes local denaturation resulting in conformational change of DNA. The binding with adenine is mostly responsible for this change. (3) The binding which breaks phosphodiester bonds. This is caused by the binding with either DNA bases and/or phosphate. The latter is stimulated by the presence of certain metal ions such as lantanium. (B) The binding characteristics were studied by using double-stranded synthetic polymers. The results supported the previously described binding forms. (C) The binding of BaP metabolites in culture cells is under investigation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To observe the structural damage of double-stranded DNA caused by the binding of various carcinogens and mutagens. The quantitative and qualitative analysis of the binding, local denaturation and breakage of polynucleotide chains is carried out using highly sensitive and accurate methods. (2) To observe carcinogenic, mutagenic and toxic effects of chemicals which interact with DNA. The physiochemical nature of bindings and biological effects are correlated.

Methods Employed: (A) Unlabeled or tritium-labeled plasmid Col E1 and SV40 DNA were incubated with ^{14}C-labeled 7,8 diol 9,10 epoxide BaP for 30 minutes at 37°C and then washed extensively with organic solvents. (B) An aliquot of DNA in Tris buffer was added with 50% formamide and spread on a surface of water using protein monolayer technique for electron microscopic observation. (C) The number of hydrocarbon molecules bound to the known number of base pairs in Col E1 (6000) or SV40 (5100) DNA was determined. (D) Gel electrophoresis was used to identify the modified (open circle, partially relaxed and linear) DNA. The extent of modification depends on the rate and nature of the binding. (E) Partially denatured sites were isolated with endonuclease S1 which specifically hydrolyzes single-stranded polynucleotides. The hydrolysate thus obtained was fractionated in Sephadex LH 20 and subjected to TLC for base analysis. (F) The endonuclease insensitive portions of DNA were hydrolysed with DNase, phosphodiesterase and alkaline phosphatase and analyzed similar to S1 hydrolysate. (G) Identical experiments were carried out using calf thymus DNA and double-stranded synthetic polymers, poly(dA-dT)(dA-dT) and poly(dG-dC)(dG-dC).

Major Findings: About 70 hydrocarbon molecules were bound covalently with a single Col E1 DNA molecule. The binding caused conformational change of the DNA in such a way that the superhelical turn is reduced thus increasing its diagonal dimension. The treatment of this DNA with endonuclease resulted in fragmentation of the DNA. The S1 sensitive sites created by the binding were estimated to be less than 10 per molecule, suggesting that only a small fraction of the binding is responsible for local denaturation of the DNA double helix. From the TLC analysis it was found that the S1 hydrolysate consisted mostly of hydrocarbon-adenine adduct, indicating that the binding with adenine is responsible for local denaturation and conformational change. The binding with guanine on the other hand did not cause base dissociation although the binding to guanine was the numerical majority (83%).

Significance to Biomedical Research and the Program of the Institute: The reaction between the 2-amino group of guanine and the 10 position of diol epoxide is generally accepted as the mechanism of covalent attachment to DNA. However, there is no evidence yet showing that this major type of binding is, in fact, the most relevant to carcinogenesis and mutagenesis. The methods used in the present study allowed analysis of the different forms of the binding and subsequent modification of DNA. Although the binding with adenine is numerically minor, it caused local denaturation of DNA double helix most extensively. If damage and subsequent repair mechanism are involved in

muta-carcinogenesis, as repeatedly mentioned in the past, the significance of adenine binding would be greater than other bindings which do not cause steric hindrance on DNA. Further investigation on this matter will provide valuable insight on the mechanism of chemical carcinogenesis.

Proposed Course of Project: (1) The modification of DNA by BaP metabolites in vivo will be carried out using hamster embryo primary culture cells. The result is compared with the in vitro binding study. (2) Adenine-diol epoxide I adduct is prepared on a large scale. Physiochemical studies to determine the binding structure will be studied using mass spectroscopic methods. (3) The binding with phosphate in relation to the breakage of phosphodiester bond and other forms of modification will be studied. (4) A number of other BaP metabolites such as K-region metabolite will be tested for possible DNA modification. The rate of DNA modification and muta-carcinogenic activities of different compounds will be correlated.

Publications

Kakefuda, T., Lovinger, G. G., Gilden, R. V., and Hatanaka, M.: Electron microscopic studies of circular DNA in mouse embryo fibroblast infected by Rauscher leukemia virus. J. Virol. 21: 792-795, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04479-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Mechanism of Induction of Metal Binding Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakefuda	Medical Officer	CH NCI
OTHER:	H. Yamamoto	Visiting Fellow	CH NCI
	M. Kimura	Senior Scientist	National Institute of Industrial Health, Kawasaki, Japan
	T. M. Bak	Microbiologist	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) The topic of the present study is the establishment of tissue culture systems for analyzing the mechanism of induction of specific proteins which covalently bind to heavy metals such as Hg, Cd, and Zn. (2) Factors required for the induction appeared to be highly complex. It depends on the new synthesis of messenger RNA and the process of protein synthesis. The level of induction of Cd binding protein is dependent upon the Ca++ uptake of the cell. The level of induction varied depending on the species of animals, organs, stages of differentiation and a number of other unknown factors. (3) The level of induction in primary culture of chick embryo cell is high, while the level of induction decreases as the cell generation passes through 16 sub-cultures. Transformation of the chick embryo cells by Rous sarcoma virus (wild-type) enhanced the induction significantly.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To observe the cellular response against heavy metals which are known to be highly toxic and potential muta-carcinogens. (2) To observe the cellular and molecular mechanisms of the induction of protein which specifically binds to the heavy metals in the cells. (3) To determine the molecular structure of protein induced in different animal cells and stages of differentiation. (4) To observe the genetic background of the induction. (5) To observe the role of metal binding protein for carcinogenesis, mutagenesis and toxicity.

Methods Employed: (A) As a standard assay system African green monkey kidney cells (VERO) in confluent culture were treated with ^{109}Cd and $CdCl_2$ (100 uC/ml, 5 ug/ml) for 16 hours. The cells were washed and homogenized by sonication and centrifuged 500 g for 4 min. The supernatant was charged on to a Sephadex G75 column. The metal binding protein was separated from other non-specific proteins. (B) To determine the dependency of the induction on the Ca^{++} ion, calcium free media with 2% serum was added with either 10 mM EGTA or different doses of $CaCl_2$. The level of induction was measured as described above. (C) The Ca^{++} uptake during induction was measured using ^{45}Ca (2 uc/ml) at different times after administration of $CdCl_2$. In other experiments calcium transport of mitochondria was blocked by rotenone, antimycin A or ruthenium red. The level of induction was then measured. (D) RNA or protein synthesis in $CdCl_2$ treated cells was blocked by actinomycin D (2 uc/ml) or by cyclohexamide (10 ug/ml). (E) A large number of primary and established culture cells were tested for their inducibility. (F) Primary chick embryo cells and those after 16 passages, Rous sarcoma virus transformed cell and temperature-sensitive mutant RSV cells were tested for inducibility at different temperatures (37° and 41°). (G) A large amount of Cd-binding protein was isolated from culture (VERO) and purified. Amino acid sequence was determined and compared with the similar protein isolated from the kidney of human patients with chronic cadmium intoxication and from other experimental animal cells.

Major Findings: (1) African green monkey kidney cells and kidney cell lines originating from other animals (mouse, mnini pig and porcine) were capable of inducing metal binding protein effectively, while other cell lines from liver or fibrobast origin were either not or weakly induced. The inducibility markedly decreased during the passage of primary culture of chick embryo cells. Transformation of such cells by Rous sarcoma virus re-activated the inducibility. (2) The inducibility is highly dependent on the level of Ca^{++} in culture media. Since Ca^{++} uptake is respiration or electron transport dependent the inducibility may also depend on the repiratory function of mitochondria. (3) The induction is completely blocked by actinomycin D or cyclohexamide. (4) The molecular structure of the binding protein induced in the culture cells was identical with those found in human cells. Some modification in amino acid composition was also detected.

Significance to Biomedical Research and the Program of the Institute: Environmental pollution by heavy metals is an increasing concern of our society because of the magnitude of their disposal by industry, their toxicity and their

potential muta-carcinogenic effects. A number of heavy metal ions such as Cd and Cr are strong carcinogens compared with the most potent organic carcinogenic compounds. Scientific information in regards to cellular and molecular events after acute and chronic intoxication is, however, relatively scarce. The tissue culture system established this past year is an extremely useful one for the investigation of this important problem.

Proposed Course of the Project: (1) Ca++ dependent induction of Cd binding protein will be further studied using refined experimental conditions. This is of particular importance since human cells of chronic Cd poisoning show severe abnormality in calcium metabolism. (2) The carcinogenic mechanism of Cr in human and experimental animal systems is almost totally unknown. The culture cells tested do not induce binding protein. The molecular events which follow after exposure to a variety of metal ions will be investigated. (3) The molecular structure of the binding protein induced in different animals, organs and culture cells under different experimental conditions will be studied by amino acid sequencing techniques.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04480-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Aryl Hydrocarbon Hydroxylase in Cultured Monocytes from Monozygotic and Dizygotic Twins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Okuda	Visiting Fellow	BGY NCI
OTHER:	E. S. Vesell	Professor	Department of Pharmacology The Pennsylvania State University College Hershey, Pennsylvania
	E. V. Plotkin	Chemist	CH NCI
	R. Tarone	Mathematical Statistician	B NCI
	R. C. Bast	Surgeon	BGY NCI
	H. V. Gelboin	Chief, Chemistry Branch	CH NCI

COOPERATING UNITS (if any)
Department of Pharmacology, The Pennsylvania State University College, Hershey, Pennsylvania

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Molecular Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	5	4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Interindividual and intraindividual variations in the basal and benz(a) anthracene-induced levels of aryl hydrocarbon hydroxylase (AHH) were investigated in cultured monocytes obtained from 10 sets of monozygotic and 17 sets of dizygotic normal adult twin volunteers. The values for basal levels, and therefore, induction ratios were more variable than those of the induced level. The mean values for the induced levels ranged from 4.26 to 17.49. Intratwin differences in the induced levels were quite small within both the monozygotic and most of the dizygotic twins. However, a few sets of dizygotic twins had much greater intratwin differences than did the monozygotic twins, providing heritability indices between 0.57 (uncorrected) and 0.71 (corrected). Thus, genetic factors accounted for approximately half to two-thirds of the inter-individual variations in AHH inducibility in this study. The small intratwin differences among most of the dizygotic twins suggests that a relatively small number of genes are involved in regulating the induced AHH levels.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine the genetic basis of aryl hydrocarbon hydroxylase differences in humans.

Methods Employed: Enzymology, Cell Culture.

Major Findings: Identical and non-identical twin pairs show very close relationships in the induced level of AHH.

Significance to Biomedical Research and the Program of the Institute: May relate to human susceptibility to polcycylic hydrocarbon carcinogenesis.

Proposed Course of the Project: To further elucidate genetic basis for AHH levels.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04516-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cellular and Molecular Effects of Psoralen Plus Ultraviolet Light

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K. H. Kraemer Research Scientist CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular and molecular effects of the combination of psoralen and long wavelength ultraviolet light (UV-A) are being studied. This combination, which induces DNA-psoralen binding is being used experimentally for treatment of psoriasis and the cutaneous lymphoma mycosis fungoides. Psoralen plus UV-A has been found to be mutagenic in bacteria and carcinogenic in mice. There is a significant decrease in DNA synthesis in circulating leucocytes from patients following this treatment. The present research involves development of an in vitro assay to measure the effects of psoralen plus UV-A on cell proliferation and DNA synthesis using rapidly dividing human lymphoblastoid cells. These studies may be useful in governing the use of psoralen plus UV-A by indicating conditions of toxicity and possible mutagenicity to human cells and in clarifying the mechanism of cell damage by psoralen.

PHS-6040
(Rev. 10-76)

Project Description

High dose oral psoralen plus high intensity long wavelength ultraviolet light (UV-A) is currently being used experimentally to induce remissions in psoriasis and in the cutaneous lymphoma mycosis fungoides. In the presence of UV-A, psoralen binds to DNA forming mono-adducts and inter-strand cross links. This combination has been shown to cause mutations in bacteria and skin cancer in mice. On the other hand, low dose psoralen with low intensity ultraviolet light has been used in humans for many years for treatment of vitiligo with no documented neoplastic sequalae. Thus, the neoplastic potential on humans of this more intense treatment is not known.

Work begun at the University of Miami and continued at NIH as part of this project has demonstrated a significant reduction in DNA synthesis in circulating leucocytes from some patients with psoriasis after treatment with psoralen plus UV-A. The immediate objective of this project is the development of an *in vitro* assay to measure the effects of psoralen plus UV-A on human leucocytes. Long term human lymphoblastoid cell lines were chosen as a model because they were derived from leucocytes, can be rapidly grown to high density in suspension culture, can initiate cultures from small numbers of cells, and have an apparently infinite life span. The effect of psoralen plus UV-A on lymphoblastoid cells is being measured in terms of changes in the rate of DNA synthesis (estimated by acid insoluble tritiated thymidine incorporation into cells harvested on Millipore filters and by autoradiography), alterations in the rate of population growth in macrocultures, and alterations in the ability of small numbers of cells to initiate microcultures. This *in vitro* assay will be used to determine the concentrations of psoralens and doses of UV-A which will inhibit DNA synthesis and cell proliferation. Different psoralen analogues may be examined to determine effects of structural modifications on these parameters. Subsequently, DNA synthesis and cell viability will be measured in short term cultures of human leucocytes treated with psoralen and UV-A *in vitro*. These studies may be useful in indicating conditions of toxicity to leucocytes in the clinical situation and in studies of the mechanism of cell killing by psoralens. Toxicity might be maximized in treating mycosis fungoides and minimized in treating psoriasis.

The proposed future course is to adapt the *in vitro* lymphoblastoid cell assay to determine if psoralen plus UV-A treatment is mutagenic to cells derived from human leucocytes. Other studies are planned to determine whether psoralen plus UV-A will induce fresh human leucocytes to become transformed into lymphoblastoid lines. These studies might indicate factors necessary for mutagenesis or transformation of leucocytes by psoralen plus UV-A. Such factors should be considered in therapy and in furthering the understanding of the mechanism of these processes.

By using lymphoblastoid cells defective in DNA repair, *e.g.* from patients with xeroderma pigmentosum, the *in vitro* assay may be modified to measure the contribution of DNA repair to the cellular and molecular effects of psoralen plus UV-A.

The lymphoblastoid cell assay system may be adapted to measure effects of other known or suspected mutagens.

Publications

Kraemer, K. H., and Weinstein, G. W.: Decreased thymidine incorporation in circulating leucocytes after treatment of psoriasis with psoralen and long-wave ultraviolet light. J. of Invest. Dermatol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04517-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

DNA Repair in Human Cancer-Prone Genetic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K. H. Kraemer Research Scientist CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Radiation and carcinogen-induced DNA damage and repair in human cancer-prone genetic diseases is being studied. Attention is presently focused on xeroderma pigmentosum (XP), a disease with ultraviolet sensitivity, and ataxia telangiectasia (AT), a disease which recently has been found to exhibit X-ray sensitivity. Cell fusion studies have demonstrated at least 5 different DNA repair defects among XP fibroblast strains. Similar studies to demonstrate possible genetic heterogeneity of DNA repair defects among AT strains are planned. A detailed comparison of the clinical features of these diseases is being made. Cell fusion studies are also planned to attempt to detect persons heterozygous for XP or AT. Correlation of clinical features of XP and AT with in vitro DNA repair defects may lead to further understanding of the link between DNA repair and cancer.

PHS-6040
(Rev. 10-76)

Project Description

Xeroderma pigmentosum (XP) and ataxia telangiectasia (AT), two rare autosomal recessive diseases associated with high incidence of neoplasia, have been found to be very sensitive to radiation and to have defective DNA repair. Studies with XP fibroblast strains performed in the Dermatology Branch, NCI, using cell fusion techniques have demonstrated 5 different defects in repair of ultraviolet-induced DNA damage. The objective of this project is to verify that AT fibroblast strains are defective in DNA repair and to determine if genetic heterogeneity exists similar to that in XP fibroblasts. Autoradiographic and cell fusion studies are planned to measure DNA repair following exposure of AT cells to X-ray or to a potent carcinogen, nitrosoguanidine.

A detailed comparison of the clinical features and cellular abnormalities in XP and AT is being made. Features common to both diseases may be the end result of extremely defective DNA repair in certain cell types or may indicate relatively greater local concentrations of DNA damaging factors. Correlation of clinical features of these diseases with in vitro DNA repair defects may lead to further understanding of the link between DNA repair and cancer.

Epidemiological studies of blood relatives of patients with AT and XP have suggested that asymptomatic carriers of these diseases have an increased incidence of neoplasia. Cell fusion studies are planned using haploid cells to attempt to detect the (heterozygous) carrier state of XP or AT in vitro. Since heterozygotes for AT or XP probably occur in frequency greater than 1%, detection of this population may be an indicator of a large population with an increased risk of cancer.

Publications

Robbins, J. H., Kraemer, K. H. and Andrews, A. D.: Inherited DNA repair defects in H. sapiens: Their relation to UV-associated processes in xeroderma pigmentosum. In Yuhas, J. M., Tennant, R. W. and Regan, J. D. (Eds.): Biology of Radiation Carcinogenesis. New York, Raven Press, 1976, pp. 115-127.

Kraemer, K. H., Andrews, A. D., Barrett, S. F. and Robbins, J. H.: Colony forming ability of ultraviolet irradiated xeroderma pigmentosum fibroblasts from different DNA repair complementation groups. Biochim. Biophys. Acta 442: 147-153, 1976.

Peck, G. L., Kraemer, K. H., Wetzel, B., Klingler, W. G. and Cohen, I. K.: Cornifying Darier's disease - a unique variant. I. Case report. Arch. Dermatol. 112: 495-503, 1976

Cohen, I. K., Kraemer, K. H. and Peck, G. L.: Cornifying Darier's disease - a unique variant. II. Surgical treatment. Arch. Dermatol. 112: 504-506, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04518-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Anticodon:Codon Adaptation and Misreading of Amino Acyl-tRNAs in Protein Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Hatfield	Research Biologist	CH NCI
OTHER:	M. Caicuts	Chemist	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine if the concentrations of isoacceptor aa-tRNAs in rabbit reticulocytes are adapted to the usage of their corresponding codons in globin mRNA (i.e., anticodon:codon adaptation) and to determine if misreading of isoacceptor aa-tRNAs in protein synthesis may play a role in the development of cancer.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To resolve isoacceptor aa-tRNAs from rabbit reticulocytes and determine the concentrations of each isoacceptor and to determine if isoacceptor aa-tRNAs which are misrecognized by mammalian aa-tRNA synthetases may transform mammalian cells in culture.

Methods Employed: Transfer RNAs are isolated by standard techniques from rabbit reticulocytes and isoacceptor aa-tRNAs are resolved by chromatography on BD-cellulose and reverse phase chromatographic columns to determine isoacceptor concentrations. Transfer RNAs are obtained from various species including E. coli, yeast and wheat germ and are aminoacylated in the presence of mammalian aa-tRNA synthetases to determine if mammalian synthetases may misrecognize heterologous tRNAs. If an isoacceptor is found which is efficiently misrecognized by mammalian aa-tRNA synthetases, this isoacceptor will be administered to mammalian cells in culture. Mammalian cells in culture are known to take-up tRNA from the media and efficient misrecognition of isoacceptor aa-tRNAs from different species has been demonstrated.

Major Findings: In an earlier project, several isoacceptor aa-tRNAs were resolved by a single run on a reverse phase chromatographic column. Codon assignments to these isoacceptor aa-tRNAs were determined and a correlation in their concentrations in reticulocytes to the frequency of usage of the corresponding codons in β-globin mRNA (Efstratiadis et al., Cell 10: 571, 1977) was observed. Other isoacceptor aa-tRNAs were not resolved by this technique and additional chromatographic techniques must be employed to determine their concentrations.

Cross aminoacylation of mammalian synthetases and fractionated E. coli tRNAs have shown that misrecognition occurs at a low level of acylation in only a few cases. Cross aminoacylation of fractionated tRNAs from other species will determine if an isoacceptor can be found which is efficiently misrecognized.

Significance to Biomedical Research and the Program of the Institute: A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. Approaches to elucidating these problems are to determine if the levels of isoacceptor aa-tRNAs are adapted to the requirements of protein synthesis; and if misreading of isoacceptor aa-tRNAs in protein synthesis can induce cell transformation.

Proposed Course of Project: To further resolve isoacceptor aa-tRNAs from rabbit reticulocytes and determine their concentrations. To determine if isoacceptor aa-tRNAs from different species can be efficiently misrecognized by mammalian aa-tRNA synthetases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04525-05 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Electrophoretic Techniques for Protein, RNA, and DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. C. Peacock	Head, Protein Section	CH NCI
OTHER:	S. L. Bunting	Chemist	CH NCI
	R. A. Reed	Biologist	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	0.8	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been undertaken to correlate the electrophoretic properties of heterogeneous nuclear RNA with other definable characteristics of these molecules such as chain length, conformation, and rates of synthesis. Although grossly similar, it appears that numerous differences exist between the group of RNAs associated with ribosomal structure and hnRNA molecules. These differences appear sufficiently great to result in marked differences in electrophoretic properties. The nature of these differences and the applicability of gel electrophoresis for the study of hnRNA are currently under study.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To investigate the influence of primary structure and conformation on the electrophoretic and biological properties of proteins and nucleic acids and to use this information in devising improved methodology for analysis and purification. To study the role of hnRNA in cellular control in normal and malignant cells by these techniques.

Methods Employed: Cultures of HeLa cells, assay of radioisotopes, isolation and electrophoresis of RNA and DNA, optical scanning of stained and unstained gels, preparative electrophoresis, optical measurement of melting of nucleic acids, ultracentrifugal analysis of RNA and DNA.

Major Findings: 1. Studies on heterogeneous nuclear RNA. Methods of RNA isolation and preparation suitable for analysis of ribosomal type RNAs were found inadequate for the study of hnRNA. The tendency of hnRNA to form aggregates of variable size gives rise to overestimates of the size of these molecules. In addition, the presence of DNA, either native or denatured, in such preparations enhances the tendency for aggregation. It has been found possible to avoid much of this difficulty by enzymatic removal of DNA, followed by denaturation of the remaining RNA in dimethylsulfoxide. The electrophoretic properties of such hnRNA preparations are highly reproducible.

It appears unlikely that the ribosomal RNA precursors used to calculate the molecular weight scale on these gels are appropriate for estimating the molecular weight of hnRNA. An independent calculation of this scale is, therefore, required. Experiments were conducted and computer simulations performed to evaluate the applicability of kinetic constants for thermal degradation in estimation of molecular weight. These studies are in progress.

Other methods for independent molecular weight determinations of the large hnRNA molecules include ultracentrifugal analysis and laser light scattering. Currently there are discrepancies between estimates of molecular weight based on centrifugal analysis and electrophoretic analysis. The resolution of these difficulties may lead to more definitive information on the size range of hnRNA.

2. Preparative electrophoresis. Yields and purity of RNA fractions isolated by preparative electrophoresis have continued to improve during the past year and currently average about 50% yield and 95% purity. Numerous difficulties persist, however, including sporadic failure for unknown reasons and the extensive time required. In addition, improvements in yield and purity have been achieved only in the presence of carrier RNAs, a feature of the method which may prove highly undesirable in many cases.

Significance to Biomedical Research and the Program of the Institute: The heterogeneous RNA is the primary transcription of the gene and, in addition to specific messengers for specific proteins, presumably contains all RNA that might be involved in the control of genetic expression. Analytical methods

for studying this RNA and assessing its role may be fundamental to understanding the alterations which occur in malignancy.

Proposed Course of Project: It is hoped to complete the analysis of the major factors that determine electrophoretic mobility of hnRNA and subsequently to examine this material in a variety of cell types and states.

Publications

Peacock, A. C., Bunting, S. L., and Nishinaga, K.: Conformational states of 18S rRNA from HeLa cells detected by gel electrophoresis. Biochim. Biophys. Acta 475: 352-365, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04554-03 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of the System for Assay of In Vitro Chemical Carcinogenesis Using Human Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakunaga	Expert	CH NCI
OTHER:	R. M. Nims	Project Director	Microbiological Associates, Bethesda, Maryland

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.6	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to establish the system of assay of in vitro malignant transformation of human cells by chemical carcinogens. The normal diploid human fibroblasts were transformed by chemical carcinogenes. Transformed cells were characterized by morphological changes in culture, growth ability in soft agar, and tumor formation in nude mice. Some conditions required for the development of the transformation were defined.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To establish a quantitative system for assay of in vitro chemical transformation of human cells.

Methods Employed: The experiences of the principal investigator with the development of a system for malignant transformation of hamster embryonic cells and A31-714 cells, a clone derived from BALB/3T3 mouse cell line, have been helpful.

The cells used have been freshly isolated from human embryo and from other normal human cell lines. Cell strains established from biopsies taken from persons who have a condition known to predispose them to malignancies, such as xeroderma pigmentosum and polyposis of the colon are also used. The cells are grown as a mass culture or as individual cells and then exposed to chemical carcinogens.

The cells are examined for the morphology and the ability to grow in soft agar. Morphology of the cells is examined by microscopy before or after fixation and staining. Cells which show altered morphology or growth patterns are picked up and tested for their ability to grow in soft agar. The colonies formed in soft agar were picked up and then retested for their growth in soft agar and were also examined for their morphology when cultured in liquid medium. The cells which show either the altered morphology or growth pattern or the ability to grow in soft agar were tested for the plating efficiency, saturation density, and tumorigenicity in nude mice. Various experimental factors including choice of serum, concentration of serum, cell density, and number of cell divisions allowed after treatment are also examined.

Major Findings: Fibroblasts (derived from biopsies taken from normal adult skin) have been successfully mass cultured. Using a selected lot of serum, cloning efficiencies of normal diploid cells as high as 60-90% have been regularly observed.

Cells treated with 4-nitroquinoline-1-oxide (4NQO) or N-methyl-N -nitro-N-nitrosoquanidine (MNNG) for various times, and incubated for 4 to 12 weeks in the medium with various kinds and concentrations of serum but without transfer of cultures did not show any morphological changes. These cells also did not show any ability to grow in soft agar when tested 2 or 6 weeks after treatment.

However, cells treated with 4NQO for 30 min in suspension and cultured with transfer of cultures at a 1:4 dilution as soon as they reached confluence did show morphological alteration such as higher cell density, loss of contact inhibition, and slight crisscross pattern of cell growth, about 6 to 12 weeks after treatment. These morphologically altered cells had abilities to grow in soft agar and to produce tumors when injected subcutaneously into nude mice. Chromosomal analysis on the cells obtained from these tumors revealed that all the tumors formed in nude mice were derived from the human cells infected. This transformation has also occurred in cultures treated with MNNG. The transformation has also been induced by treating a clone of normal diploid human fibroblast with 4NQO.

The number of cell divisions required for the development of the transformation was calculated approximately 12-14 after 4NQO-treatment.

Significance to Biomedical Research and the Program of the Institute: To date there has been no reproducible system established in which normal diploid human cells are transformed in vitro by chemicals. Rationale for the establishment of such a system is as sound as any at this time. This project will provide a system in which to assay potential carcinogens directly on human cells, thus removing the necessity of extrapolating from data obtained from animal cells. It would also provide important information on the relationship of the response to the carcinogenic effect of chemical compounds between the cells from different species and between in vivo and in vitro results. The information thus obtained would be very helpful for extrapolating the result in animal experiments or in in vitro system into assessment of human risk to environmental carcinogens.

Proposed Course of Project: To pursue the goals as outlined above in Objectives and to publish any results so obtained.

Publications

Kakunaga, T.: The transformation of human diploid cells by chemical carcinogens. In Watson, J. D. (Ed.): The Origin of Human Tumor. New York Cold Spring Harbor Laboratory, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04555-03 CH

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Factors Effecting Malignant Transformation by Chemical Carcinogenesis in Human and Mouse Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakunaga	Expert	CH NCI
OTHER:	K. Lo	Senior Staff Fellow	CH NCI
	K. Ohki	Visiting Fellow	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Chemistry Branch, Carcinogenesis Program

SECTION

Cell Growth Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this work to clarify the mechanism of cell transformation by studying the genetic, biological, and biochemical factors affecting carcinogenesis by chemical carcinogens. This work also aims to clarify the role of these factors in the etiology of human cancer incidence. The topics of present interest are: (1) the effects of physiological conditions of cells on the transformation; (2) the mechanism of fixation of transformation, in particular the role of DNA repair; and (3) the characterization of cell variants which shows the different sensitivity to chemical transformation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To learn more about the mechanisms involved in the malignant transformation of cells in vitro and to determine more appropriate systems for the in vitro assay of chemical carcinogens.

Methods Employed: Two systems of cell transformation by chemical carcinogens were used: (1) A quantitative system for the assay of malignant transformation of subclones derived from BALB/3T3 clone which was previously developed (Int. J. Cancer 12: 463-473, 1973). (2) A system of malignant transformation of human diploid fibroblast derived from the skin biopsy obtained from a normal female adult which was developed recently (see Project No. Z01-CP-04554-03-CH). Culture conditions, genetic factors, and physiological state affecting the transformation frequency were examined. These included the kinds and concentrations of serum, the growing state of cells, dell density, phase of cell cycle, and the isolation and use of mutant cell lines which show different susceptibility to chemical carcinogens. The amount of 4-nitroquinoline-1-oxide (4NQO) bound to DNA base were analyzed according to the method by T. Kakunaga (Cancer Res. in press).

Major Findings: 1) In the transformation of human diploid cells by 4NQO, it was found that approximately 12-14 cell generations were required for the development of transformation. No transformation was observed when the treated cells were maintained at the nongrowing state after 4NQO-treatment. This requirement of cell division for the transformation has also been observed in the transformation of mouse cells as described previously (see Project No. Z01-CP-04555-02-CH).

2) By using synchronized population of mouse cells, it was found that the cells in the S-phase or in the late G -phase were the most susceptible to the 4NQO-transformation. The inhibitory effect of caffeine on the 4NQO-transformation was also proved to be dependent on the first S-phase after 4NQO-treatment.

3) Short exposure of the cells to 4NQO resulted in the covalent binding of 4NQO to DNA, mainly guanine and adenine base. These 4NQO-adducts were removed from the DNA strand during post-incubation even at the nongrowing state. This removal of 4NQO-adducts were observed in mouse cells and normal human fibroblast cells but not in the cells derived from classical type of xeroderma pigmentosum, suggesting that 4NQO-adducts are removed out through the mechanism of DNA repair similar to that which excises thymidine-dimer produced in DNA by UV-irradiation. The decrease in the amount of 4NQO-adducts in DNA during post-incubation at the nongrowing state was parallel to the loss of fixation of the transformation under the same conditions. These results combined with other findings support the hypothesis that repairable DNA damage is the source of the cell transformation and that caffeine-inhibitable repair which occurs at S-phase plays an important role in the process of the transformation by chemical carcinogens.

4) Cell mutants, isolated from a clone of BALB/3T3 cell line (see Project No. Z01-CP-04555-02) showed different susceptibility to the transformation. The

difference in the susceptibility between mutants was common to the transformation by all carcinogens tested, such as benzo(a)pyrene, 3-methylcholanthrene, 4NQO, N-methyl-N -nitro-N-nitrosoguanidine, and ultraviolet. In contrast, the sensitivity to the cytotoxic effect of these agents were not markedly different between these mutants. There was no significant difference in the inducibility of arylhydrocarbon hydrase activity in response to treatment with benzanthracene between these mutants. These results suggest that these mutants may differ in the functions which are directly involved in the mechanism of cell transformation by physical and chemical agents.

Significance to Biomedical Research and the Program of the Institute: The results with this project will provide: 1) A useful system for the analysis of the mechanism of chemical carcinogenesis. 2) Information about the mechanism of cell transformation by chemical carcinogens. 3) Optimum conditions needed for the assay of potential chemical carcingens using human and mouse cells. 4) Basic information about the etiological factors in the incidence of human cancer. Thus, this project is directly aimed at that aspect of the Program of the Institute which seeks to find rapid meaningful assays for potential chemical carcinogens.

Proposed Course of Project: To pursue the goals as outlined in Objectives and to publish any results so obtained.

Publications

Dingman, C. W. and Kakunaga, T.: DNA strand breaking and rejoining in response to ultraviolet light in normal human and xeroderma pigmentosum cells. Int. J. Radiat. Biol. 30: 55-66, 1976.

Ikenaga, M. and Kakunaga, T.: Excision of 4-nitroquinoline-1-oxide damage and transformation in mouse cells. Cancer Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04563-04 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies with a Cationic Carbocyanine Dye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Green Research Chemist CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methyl-propenyl]naphtho[1,2d]thiazolium bromide is a metachromatic dye of unusual sensitivity for differentiating various classes of macromolecules by color. Methods for staining proteins, phosphoproteins, sialoglycoproteins, glycosaminoglycans and nucleic acids differentially in tissue sections and on polyacrylamide gels following electrophoresis were developed. These methods were applied to the study of milk and breast tissue proteins. A histochemical study of normal and neoplastic human breast tissue was done and the macromolecules listed above were identified.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To obtain more information about the characteristics of the dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methylpropenyl]-naphtho[1,2d]thiazolium bromide and its interaction with macromolecules. Identification of acidic macromolecules on gels and tissue sections by metachromatic reactions.

Methods Employed: Polyacrylamide gel electrophoresis, staining and scanning of gels with a spectrophotometer, absorption measurements of dye solutions in a recording spectrophotometer, observation of color reactions of pure substances in films and solutions, enzymatic digestions and solvent extractions of cryostat sections, microscopic observation of stained sections.

Major Findings: Phosphoproteins and sialoglycoproteins were identified among other proteins in as little as 1 µl of milk and on polyacrylamide gels of separated proteins derived from 3 mg wet weight of breast tissue. The simultaneous differential staining of phosphoproteins, sulfated glycosaminoglycans, hyaluronic acid, sialoglycoproteins, and nucleic acids was demonstrated in surgical specimens of normal and malignant human breast tissue fixed in formalin and embedded in paraffin using the dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2d]thiazolium bromide ("Stains-all"), a metachromatic cationic carbocyanine dye. This simple staining method gives information concerning the types of macromolecules present in connective tissue and in secretions within cells, alveolar lumina and ducts. Precise relationships among various tissue elements and macromolecules are seen. Nuclear counterstaining is not required. Enzymatic digestions with Streptomyces hyaluronidase confirmed the presence of variable but easily detectable amounts of hyaluronic acid in the connective tissue stroma of several cases of breast carcinoma. Hyaluronic acid was also in the intralobular stroma of a fibroadenoma from a pregnant individual. Neuraminidase digestion confirmed the presence of sialoglycoproteins in alveolar lumina, within ducts and, in some carcinomas, within cells. Sialoglycoproteins were present in many of the malignant breast tissues examined. These macromolecules as well as phosphoproteins were easily detectable because of color difference from pink cytoplasm and purple nuclei. Comparison with Alcian Blue, Periodic Acid Schiff or Toluidine Blue staining indicates that "Stains-all" has advantages in terms of simplicity, specificity and sensitivity for the detection of these macromolecules in pathologic material.

Significance to Biomedical Research and the Program of the Institute: This stain is particularly useful for detection of sialoglycoprotein production by normal and malignant tissue and for showing the presence of hyaluronic acid and sulfated glycosaminoglycans in stromal tissue. It would be of interest to correlate the presence of sialoglycoprotein in the primary tumor with the presence in serum of CEA, κ casein and β subunit of HCG resulting from recurrent metastases. If such a correlation could be made, it might help in selecting patients for such radioimmunoassay testing. The determination of the types and kinds of macromolecules synthesized by normal and neoplastic tissue is important for many reasons. A technique which can easily identify

different classes of macromolecules in small samples without the use of radioactive precursors is useful toward achieving such goals.

Proposed Course of Project: The histochemical methods will be applied to cytologic and biopsy specimens to determine whether they have special utility for the detection of early neoplastic lesions. Normal and neoplastic cells in culture will be studied to determine whether the production of certain macromolecules can be correlated with neoplastic transformation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04578-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genes Responsible for the Transformed Phenotype in Chemically Transformed Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Ohki	Visiting Fellow	CH NCI
OTHER:	T. Kakunaga	Expert	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to clarify the nature of molecules responsible for converting a normal cell to its malignant form by studying the nature of temperature-sensitive (ts) or cold-sensitive (cs) mutants of Chinese hamster lung cells affected in the expression of the transformed state. Several ts- and cs-sensitive mutants were isolated and confirmed to show the normal phenotype at non-permissive temperature and the transformed phenotype at permissive temperature. The resistance to 6-thioguanine or ouabain was introduced into these mutants for further studies.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To learn about genetic information on the expression of transformed phenotype, and to obtain the clues for biochemical approach to the molecules controlling the transformed phenotype.

Methods Employed: In ts-mutants, permissive temperature of the incubation is 34.5°C and non-permissive temperature is 39°C for the incubation. Permissive temperature is 30°C and non-permissive temperature is 34.5°C in cs-mutants. Ability of the cells to grow in soft agar (Int. J. Cancer 12: 463-473, 1973) is used as a transformed phenotype because of its close correlation to the in vivo malignancy of the cells, and because of its more objective process to diagnose this criteria compared to other criteria of the transformed phenotype.

A cell hybridization technique is used for examining the complementation group of mutants. In order to select out the hybrid cells from nonhybrid cell population in this experiment, markers like a resistance to 6-thioguanine and/or ouabain is introduced into all mutants. Polyethylene glycol is used to induce the cell fusion. Optimum experimental conditions are determined to obtain 1:1 hybrid cells at high frequency.

Major Findings: The isolated mutants were confirmed to grow in soft agar at permissive temperature and not at non-permissive temperature. Growth in liquid medium is not affected markedly by the different temperature of incubation. Many clones which were resistant to both 6-thioguanine and ouabain were isolated from each ts- and cs-mutants. These double drug resistant clones were confirmed to retain their original temperature-dependency to express the transformed state. Optimum conditions for the cell hybridization and the selection of 1:1 hybrid were determined with the density and growth state of cells, concentration, and duration of polyethylene glycol-treatment, the time between cell fusion and addition of selective agent, and the concentration of selective agent.

Significance to Biomedical Research and the Program of the Institute: The results with this project will provide: 1) A useful system for the analysis of the transformed phenotype; 2) The information about genes controlling the expression of the transformed state; 3) Information on the nature of the molecules responsible for converting a normal cell to a malignant cell. Thus, this project is directly aimed at that aspect of the Program of the Institute which seeks to find an essential difference in the biochemical nature between normal and malignant cells.

Proposed Course of Project: To pursue the goals as outlined in Objectives and to publish any results so obtained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04579-01 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Benzo(a)pyrene and (-)trans-7,8-diol Metabolism by Highly Purified Forms of Cytochrome P-450.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Deutsch	Visiting Scientist	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI
	J. C. Leutz	Chemist	CH NCI
	M. J. Coon	Professor	U. of Michigan School of Medicine Ann Arbor, Michigan

COOPERATING UNITS (if any)
Department of Biological Chemistry, University of Michigan School of Medicine, Arr Arbor, Michigan

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Molecular Carcinogenesis

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the mechanism of the activation and detoxification of benzo(a)pyrene by mixed function oxidase. For this purpose highly purified cytochrome P-450's from rabbit liver in a reconstituted system were used for the metabolism and DNA binding of benzo(a)pyrene and (-)trans-7,8-diol.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine the metabolism of carcinogenic polycyclic hydrocarbons by purified forms of cytochrome P-450, the principal enzymes involved in carcinogen activation and detoxification.

Methods Employed: Fluorospectrophotometry, high pressure liquid chromatography and masspectrometry.

Major Findings: Highly purified cytochrome P-450's from rabbit liver in a reconstituted system exhibit different catalytic activities in the metabolism of benzo(a)pyrene and BP(-)-trans-7,8-diol. The different cytochrome P-450's also exhibit differences in the activation of BP and (-)trans-7,8-diol to highly mutagenic and carcinogenic diol epoxide I and II.

Significance to Biomedical Research and the Program of the Institute: The analysis of the selectivity of the multiple cytochrome P-450 forms is an essential step toward the understanding of chemical carcinogen action. The unique catalytic activity of different cytochrome P-450's may be the basis between activation and detoxification pathways of benzo(a)pyrene. This finding is an essential step toward the understanding of chemical carcinogen action, and may help in the modification of the body's defense against chemical carcinogens.

Proposed Course of Project: To identify and quantitate the benzo(a)pyrene metabolites formed by the multiple forms of cytochrome P-450. To determine the role of the enzyme forms in the biological effects of benzo(a)pyrene in toxicity, mutagenicity and carcinogenicity.

Publications

Deutsch, J., Leutz, J.C., Yang, S.K., Coon, M. and Gelboin, H.V.: Different catalytic activities of two highly purified cytochrome P-450(LM)s in the metabolism and DNA-binding of benzo(a)pyrene and (-)trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene. 68th Annual Meeting of the American Association for Cancer Research (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04586-02 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetic and Epigenetic Regulation of Mixed-Function Mono-oxygenases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. J. Wiebel	Senior Staff Fellow	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI
	S. Brown	Fogarty Visiting Fellow	VA Hospital Washington, D.C.
	J. D. Minna	Chief, Oncology Branch	VA Hospital Washington, D.C.

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Molecular Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The activities of the microsomal mixed-function mono-oxygenases detoxifying and activating carcinogenic polycyclic hydrocarbons are key factors in the initiation of chemical tumorigenesis. The constitutive level and the inducibility of these enzymes are under tight genetic control but are also subject to modification by exogenous chemicals, i.e., inducers and inhibitors. It is the aim of this project to study the regulatory mechanism of enzyme activity in cells in culture, to dissect the genetic components of regulation in somatic cell hybrids and to explore the mechanism of induction by exogenous compounds. It has been found that the AHH structural and inducer genes are associated with the human chromosome number two.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To analyze the genetic and epigenetic regulatory mechanisms of microsomal mono-oxygenase activity in mammalian cells in culture.

Methods Employed: Somatic cell hybridization, fluorospectrophotometry, gel electrophoresis.

Major Findings: The activity of the mixed-function oxygenase, aryl hydrocarbon hydroxylase, was assigned to a human chromosome number two by comparing the relationship to the expression of human isozymes in somatic mouse-man cell hybrids. The results suggested that the gene(s) regulating the rate of synthesis of the oxygenase is in close proximity to the structural gene and that both activities are on the same chromosome.

Significance to Biomedical Research and the Program of the Institute: The enzyme systems studied are involved in the metabolism of numerous drugs, carcinogens and environmental pollutants, and are pivotal factors in the response of the human organism to potentially toxic and carcinogenic foreign compounds.

Proposed Course of Project: To continue the study of the elements of genetic control of human mixed-function oxygenase activity and the mechanism of induction in somatic cell hybrids.

Publications

Brown, S., Wiebel, F., Gelboin, H. and Minna, J. D.: Assignment of locus required for flavoprotein-linked monooxygenase expression to human chromosome 2. Proc. Natl. Acad. Sci. 73: 4628-4632, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04595-02 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Mechanism of Induction of Aryl Hydrocarbon Hydroxylase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. R. McIntosh	Visiting Fellow	CH NCI
OTHER:	A. C. Peacock	Head, Protein Section	CH NCI
	H. V. Gelboin	Chief, Chemistry Branch	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The activity of the aryl hydrocarbon hydroxylase of rat liver and of cultured rat liver cells is stimulated by pre-exposure of the rat or cultured cells to particular carcinogens. It is the purpose of this project to investigate the mechanisms by which this induction occurs. As the first stage of this investigation, electrophoretic estimations were made of the levels of the various microsomal proteins in rat liver, after treatment of the rats with the carcinogenic polycyclic hydrocarbon, 3-methylcholanthrene. These results were compared with those found after phenobarbital treatment. With the carcinogen two microsomal proteins were induced concomitantly in several strains of rat, and there was evidence suggesting that a third microsomal protein was decreased in amount. Increases in some microsomal proteins were also found after phenobarbital treatment, but these proteins were of somewhat lower molecular weight than those produced in response to the carcinogen. Similar investigations of the induction process are now being made in vitro, in slices of rat liver and in cultured diploid rat liver cells.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To elucidate the mechanism of the induction of aryl hydrocarbon hydroxylase activity in rat liver and cultured rat liver cells by carcinogens.

Methods Employed: Currently, the multiple forms of cytochrome P-450 in rat liver are being separated by electrophoretic techniques.

Major Findings: Clear differences in the composition of liver microsomal proteins from carcinogen-treated and untreated rats have been distinguished and the kinetics of appearance of carcinogen-induced microsomal proteins examined in several strains of rat.

Significance to Biomedical Research and the Program of the Institute: A knowledge of the induction process is central to a better understanding of the mechanism of chemical carcinogenesis.

Proposed Course of Project: It is proposed to investigate whether the increased accumulation of a particular P-450 species is the rate-limiting event in the induction of aryl hydrocarbon hydroxylase activity, and whether such accumulation is the result of an increase in the net rate of *de novo* synthesis of this species or due to the metabolic-conversion of a pre-existing P-450 species. Using the same electrophoretic methods, it is intended to attempt to characterize the microsomal proteins induced in human peripheral blood cells by polycyclic hydrocarbons.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04597-02 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Identification of the Ultimate Carcinogenic Form of Benzo[a]pyrene

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. K. Yang	Staff Fellow	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI
	D. W. McCourt	Chemist	CH NCI
	P. P. Roller	Research Chemist	CMT NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Molecular Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to find out what the ultimate carcinogenic metabolite of benzo[a]pyrene is. The steps toward this goal are: (1) to understand the detailed metabolic profiles of benzo[a]pyrene formed by microsomal mixed-function oxidases and epoxide hydratase; (2) to determine the chemical identity of the metabolites; (3) to elucidate the mechanism of enzymatic activation; (4) to synthesize the identified metabolites in large quantity; (5) to test the carcinogenic and mutagenic activity of the synthesized metabolites.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To develop analytical methods for detection of secondary metabolites of benzo[a]pyrene; (2) to determine the structure and property of the metabolites; (3) to test the carcinogenic and mutagenic activity of the metabolites; (4) to develop an assay for the detection of carcinogenic metabolites; (5) to elucidate the detailed mechanism of enzymatic activation of benzo[a]pyrene.

Methods Employed: Gravity-flow and high-pressure liquid chromatography, UV and fluorescence spectrometry, mass spectrometry, liquid scintillation counting, organic systemsis.

Major Findings: A combination of gravity-flow and high-pressure liquid chromatography has been developed to separate all the primary benzo[a]-pyrene metabolites. A high-pressure liquid chromatographic system has been developed to analyze secondary metabolites of benzo[a]pyrene. The detailed mechanism of the enzymatic formation of a highly mutagenic metabolite, r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene has been elucidated which may be the ultimate carcinogenic form of benzo[a]pyrene. This diol-epoxide is also the most potent carcinogen among BP derivatives in experimental animals.

Significance to Biomedical Research and the Program of the Institute: The identification of ultimate carcinogenic form of benzo[a]pyrene is of prime importance in the prevention of cancer caused by benzo[a]pyrene.

Proposed Course of Project: To further identify and determine the muta-genicity and carcinogenicity of benzo[a]pyrene metabolites.

Publications

Yang, S.K. and Gelboin, H.V.: Microsomal mixed-function oxidases and epoxide hydratase convert benzo[a]pyrene stereospecifically to optically active dihydroxydihydrobenzo[a]pyrenes. Biochem. Pharm. 25: 2221-2225, 1976.

Yang, S.K. and Gelboin, H.V.: Nonenzymatic reduction of benzo[a]pyrene diol-epoxides to trihydroxypentahydrobenzo[a]pyrenes by reduced nicotinamide adenine dinucleotide phosphate. Cancer Research 36: 4185-4189, 1976.

Yang, S.K., McCourt, D.W., Roller, P.P. and Gelboin, H.V.: Enzymatic conversion of benzo[a]pyrene leading predominantly to the diol-epoxide r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene through a single enantiomer of r-7,t-8-dihydroxy-7,8-dihydrobenzo[a]pyrene. Proc. Natl. Acad. Sci. 73: 2594-2598, 1976.

Yang, S.K., Gelboin, H.V., Weber, J.D., Sankaran, V., Fischer, D.L. and Engel, J.F.: Resolution of optical isomers by high-pressure liquid chromatography. The separation of benzo[a]pyrene trans-diol derivatives. Anal. Biochem. 78: 520-526, 1977.

Yang, S.K., Gelboin, H.V., Trump, B.F., Autrup, H. and Harris, C.C.: Metabolic activation of benzo[a]pyrene and binding to DNA in cultured human bronchus. Cancer Research 37: 1210-1215, 1977

Yang, S.K., McCourt, D.W., Leutz, L.C. and Gelboin, H.V.: Benzo[a]pyrene diol epoxide: Mechanism of enzymatic formation and optically active intermediates. Science (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04598-02 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

DNA Repair Deficiency and Increased Probability of Cancer in Certain Human Genetic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. A. Scudiero Senior Staff Fellow CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study chemical carcinogen-induced DNA repair in cell lines derived from patients with various genetic diseases showing an enhanced frequency of malignancy. Such investigations can determine if these cells have deficiencies in their ability to repair DNA damage and if such deficiencies can be correlated with the increased probability of cancer in these patients. Also, these studies can yield information about different classes of DNA-lesions induced by chemical carcinogens and whether or not the type of lesion induced can be correlated with the ability of different cell lines to repair damage induced by particular chemical-agents.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To examine the DNA repair capacities of cell lines derived from persons with various genetic diseases in which there is high probability for cancer. Specifically, to measure repair synthesis in these cells after treatment with a variety of chemical carcinogens.

Methods Employed: A simple methodology utilizing benzoylated naphthoylated DEAE cellulose chromatography (BND-cellulose) for the rapid quantitative estimation of excision repair and the rigorous separation of repair synthesis from semi-conservative replicative synthesis has recently been developed. In the presence of hydroxyurea, an inhibitor of semi-conservative DNA synthesis, the progression of DNA chains is slowed and isotope incorporated by residual replicative synthesis will remain in the growing point region. Because the growing point contains single-stranded regions, DNA containing the newly incorporated isotope will adhere to the column and require formamide for elution. Isotope incorporated by repair synthesis into the bulk of the DNA (double-stranded) will be eluted with native DNA in the 1.0M NaCl eluate. After corrections for pool size are made, the specific repair activity induced by any treatment can be calculated from the radioactivity and absorbancy at 260nm of the 1M NaCl eluate. By utilizing this method, agents which induce large-patch (UV-like) repair synthesis can easily be distinguished from those inducing small-patch (x-ray-like) repair synthesis. Treatment of cells with radioactive-labeled carcinogens can yield useful information about the reactivity of chemical carcinogens with cellular DNA. The amount of DNA binding and excision can be measured for a variety of chemical agents using different cell lines, and the amount of repair synthesis per lesion excised can be determined.

Major Findings: The BND cellulose technique for measuring DNA repair synthesis was used to demonstrate that r-7, t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol-epoxide I), the most mutagenic metabolite of benzo(a)pyrene yet isolated, induced approximately 20-fold more repair synthesis in "normal" human fibroblast cell lines than in excision-repair deficient cell lines derived from patients with xeroderma pigmentosum (XP). A similar difference was observed after treatment with ultraviolet light (UV). Using labeled diol-epoxide I, carcinogen-binding to DNA was examined. It was found that diol-epoxide I was excised from DNA of the "normal" human fibroblast cell line - KD upon post-treatment incubation; whereas, XP fibroblasts remove much less of the initially bound carcinogen (1 uM diol-epoxide I: 85% removed from KD in 4 hours, 20% removed from XP). These data were consistent with the repair synthesis results. These results will be reported in a publication presently in preparation.

Repair synthesis was measured in several different cell lines after treatment with a variety of chemical agents. Cell lines derived from normal individuals, and from patients with two autosomal recessive disorders with a greater predisposition to cancer than the general population: Xeroderma pigmentosum (deficient in excision repair of UV-like damage) and ataxia telangiectasia

(AT) (more sensitive to x-ray damage and deficient in enzymatic DNA repair active on x-ray modified base residues). All three cell types: normal, XP, and AT, demonstrated similar levels of repair synthesis following treatment with the monofunctional alkylating agent, methyl methanesulfonate (MMS). Several cell lines of each type have been tested. Normal and AT cell lines exhibited control-high levels of repair synthesis following UV treatment; whereas, XP cells show low levels of repair. These control levels of repair synthesis were over 5 times greater than those observed after MMS treatment. This difference is typical of repair induced by large- and small-patch repair inducing agents. When treated with N-methyl-N'-nitro-N-nitroso-quanidine (MNNG), normal cells showed a level of repair synthesis intermediate between those observed after UV- and MMS- treatment. After MNNG-treatment, XP cells showed slightly depressed levels of repair synthesis, and AT cells showed 2-5 times less repair synthesis than did normal cell lines. Previously, the only defect observed in the AT repair-response was sensitivity to x-rays. The results of these experiments will be reported in a publication presently in preparation.

Repair synthesis following treatment with chemical carcinogens is also being studied in cell lines derived from patients with other genetic disorders with an enhanced frequency of malignancy: Fanconi's anemia, Down's syndrome, and familiar melanoma.

Experiments are being initiated to further characterize the cellular response to treatment with MNNG. It has previously been shown that excision repair induced by MNNG occurs to a greater extent in growing point regions of the DNA of a human lymphoblastoid cell line than in the bulk of the DNA, even though the overall amount of MNNG-induced alkylation is the same for replicating and non-replicating regions of the DNA. Because of the enhanced sensitivity of AT and XP cells to MNNG, it is of interest to examine whether or not these cell lines exhibit a similar differential growing point-repair response. Such experiments are presently underway.

Significance to Biomedical Research and the Program of the Institute: Present evidence indicates that chemical carcinogens exert their carcinogenic and mutagenic effects by alterations in cellular DNA. The manner in which cells recognize and eliminate DNA damage is important in assessing an agent's potential for cancer induction. A systematic study of genetic diseases with both a high incidence of cancer and a diminished capacity for DNA repair can lead to useful information concerning the etiology of cancer and the role of DNA repair protecting the organism from potential carcinogenic damage.

Proposed Course of Project: To pursue the goals outlined in Objectives and to publish these results.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04782-07 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Hormones and Breast Tissue Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. R. Green	Research Chemist	CH NCI
OTHER:	J. V. Pastewka	Chemist	CH NCI
	A. C. Peacock	Head, Protein Section	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to contribute to the understanding of hormone action on metabolic processes in breast tissue under normal conditions and to understand the failure of or aberrant response to hormones under abnormal conditions. Among the topics studied are the response of mammary tissue in culture to hormones in terms of nucleic acid, protein, glycoprotein and glycosaminoglycan synthesis. The kinds of proteins made by mammary tissue from normal non-pregnant, mid-pregnant, lactating animals and by neoplastic mammary tissue are being analyzed. Response to prolactin and steroid hormones is assessed by chemical and histochemical methods.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To gain a clearer understanding of the interaction of hormones with breast tissue. The topics of interest are interaction of hormones such as insulin, hydrocortisone and prolactin with breast tissue components and metabolism of steroids by breast tissue. Responses by breast tissue to prolactin and steroids in terms of nucleic acid synthesis and synthesis of macromolecules such as phosphoproteins, sialoglycoproteins, glycosaminoglycans and proteins are under investigation.

Methods Employed: Culture of mammary tissue explants *in vitro* using defined medium, isolation and characterization of nucleic acids, separation on agarose-acrylamide gels and on gradients, radioactive tracer techniques, isolation and purification of proteins from milk and explants, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, histochemical analysis of tissue sections, autoradiography, binding of hormones to macromolecules, mutagenesis assay using Salmonella tester strains.

Major Findings: Caseins from mice were characterized and analyzed in sodium dodecyl sulfate-polyacrylamide gels at neutral pH. Four caseins were separated electrophoretically in gels varying from 8.5 to 15% acrylamide. Molecular weights for three of these proteins were 43,000, 27,700 and 25,900. A fourth mouse casein was found to contain carbohydrate, phosphorus, and sialic acid. Caseins were identified among other breast tissue proteins in mice at mid-pregnancy. Densitometric analysis of stained milk proteins made in response to prolactin by mammary explants in culture was performed following electrophoresis on SDS gels of soluble explant proteins. Caseins were detected following a one-day incubation of explants with ovine prolactin. The casein produced following a five-day incubation was used as a bioassay for prolactin.

A series of investigations was initiated to determine whether steroids may be metabolized to mutagenic derivatives under certain circumstances. Steroids tested with and without S-9 supernatants from Sprague-Dawley male and female rats using the Ames test were estrone, estriol, estradiol, testosterone, progesterone, 2-hydroestrone and 2-hydroxyestradiol. Diethylstilbestrol and dimethylbenzanthracene were also tested. The results so far indicate that steroids produced no more than twice the number of revertants than control plates with S-9 alone. Further testing is planned with emphasis being given to the estrogens. Binding of tritiated androstenedione and estradiol to DNA *in vitro* following incubation with microsomes was studied. Further binding experiments using *in vitro* incubations with DNA are planned.

Significance to Biomedical Research and the Program of the Institute:
Bioassay for Prolactin: Prolactin is a key hormone for milk production. It has been implicated as playing a role in the development of breast cancer in animals. Its role in the development of human cancers of the breast and prostate is not clear. The method of isolation of prolactin from human pituitaries produces several species of hormone varying in size and charge. It is important to know which species are biologically active. Because the

amounts obtained are limited, sensitive assays for small amounts of material are required. The bioassay described fulfills this need.

The influence of hormones on human breast cancer has been the subject of intensive investigation. Epidemiologic investigations of recent years have tended to reinforce the idea that genetic and environmental factors enter heavily into this disease process. An increased association has been shown among individuals migrating from the Orient to Hawaii and then to the United States. Dietary factors and obesity as well as age at first pregnancy play an important role. Since metabolic processes seem implicated, one wonders whether under unfavorable circumstances hormones of a certain class may become carcinogens. Estrogens given over long periods of time in large quantities have proved carcinogenic in animals. The association in humans is less clear. The Sprague-Dawley rat has been the animal of choice for the study of DMBA mammary tumorigenesis. Prior to this time it was known that this strain was subject to spontaneous mammary cancer. The S-9 supernatant of this strain has been used in the Ames assay, The understanding of the molecular basis of binding of carcinogens like BP and DMBA to DNA is increasing at a very rapid pace. Since there are similarities in molecular structure to steroids, it becomes of great interest to reinvestigate the latter with newer, more sensitive techniques.

Proposed Course of Project: Studies of synthetic processes of mammary explants as influenced by hormones *in vitro* will continue.

The interaction of steroids, such as estrogens, and possible estrogen precursors and metabolites following metabolic activation by liver and breast fractions with DNA, will be assessed by using the Salmonella revertant strains. Radioactive labeled steroid binding to DNA *in vitro* will be studied.

Publications

Green, M. R. and Pastewka, J. V.: Molecular weights of three mouse milk caseins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and κ-like characteristics of a fourth casein. J. Dairy Sci. 59: 1738-1745, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04785-07 CH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Role of DNA Repair Mechanisms in the Etiology of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. S. Day, III Research Physical Scientist CH NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The adenovirus host-cell reactivation assay, in which adenovirus 2 or 5 is treated with physical or chemical agents, and subsequently assayed for biological activity on monolayers of human fibroblasts, either capable of repairing ultraviolet damaged DNA or not, was used to study the action of several compounds on DNA. (Agents that make DNA damage repairable by ultraviolet repair mechanisms reduce the plaquing ability of adenovirus more rapidly if repair deficient (xeroderma pigmentosum) fibroblasts are used as viral hosts, than if normal ones are used.) Benzo[a]pyrene diol-epoxide I, the probable carcinogenic form of benzo[a]pyrene, when dissolved in tetrahydrofuran was found to inactivate adenovirus 5 30-fold more rapidly when the treated viruses were grown in xeroderma pigmentosum fibroblasts than when they were grown in normal fibroblasts. Moreover, one diol-epoxide I molecule bound to one virus appears to inactivate that virus when it infects xeroderma pigmentosum fibroblasts. A new ultraviolet light effect on the lambda phage - E. coli system was discovered, and a mutant of adenovirus 2 unable to grow in normal human fibroblasts, but able to grow in human tumor cells was discovered.

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Objectives: To learn more about DNA repair mechanisms in human cells and about their role in carcingenesis. In particular, to determine the nature of the biochemical defects in human cell strains grown from biopsies taken from persons having extreme susceptibility to carcinogenesis, and to determine the biological consequences of the repair defects of these cells.

Methods Employed: Using an adenovirus-host cell reactivation assay developed previously in this project, it has been possible to quantitate the deleterious effects of various chemical and physical treatments on the ability of the virus to initiate and sustain infection. The method involves establishing a monolayer fibroblast culture which is then infected with treated adenovirus. The infected cells are then incubated 17-20 days with feeding by means of periodic overlaying with a nutrient agar. Non-treated virions or those treated ones which have been "reactivated" by fibroblast cell repair mechanisms form countable plaques of dead, lysed cells. The difference in survival of treated virus measured using normal cells, (proficient in repair of DNA damage due to 254nm ultraviolet irradiation) from that measured using cells from patients having xeroderma pigmentosum (deficient in such repair) is used as our index of the amount of repairable DNA damage produced by the physical or chemical treatments. The plaquing efficiency of unirradiated adenovirus is approximately the same on all of the non-transformed human cell strains so far tested. It is 5 to 100-fold greater when human tumor lines of various types are used. Neutral and alkaline sucrose gradients are used to analyze the size of DNA extracted from treated adenoviruses.

Major Findings: 1) Studies concerning the anti-adenoviral action of the suspect carcinogenic form of benzo(a)pyrene, 7,8 dihydroxy 9,10 epoxy, 7,8,9, 10 tetrahydro benzo(a)pyrene (diol-epoxide I) have been completed. Adenovirus 5, treated with diol-epoxide I loses plaque forming ability 30-fold more rapidly if treated virions are used to infect fibroblasts from certain xeroderma pigmentosum patients than if used to infect normal fibroblasts. This result parallels findings obtained when the virus was inactivated by ultraviolet light. Furthermore, analysis of the number of diol-epoxide I molecules bound to viral DNA with increasing diol-epoxide I suggests that one diol-epoxide I molecule bound per virion is sufficient to inactivate the virus when infecting the xeroderma pigmentosum fibroblasts. This finding indicates that XP fibroblasts are probably totally deficient in the repair of diol-epoxide I damaged adenovirus 5, and strongly suggests that the same cellular mechanism is responsible for repairing both ultraviolet light and diol-epoxide I produced DNA damage. 2) During the above experimentation it was found that benzo(a)pyrene 4,5 oxide in tetrahydrofuran (as a solvent) inactivated adenovirus 5, but that the same compound in dimethylsulfoxide solvent did not. This indicated the possibility of using tetrahydrofuran to "open-up" the protein protected adenovirions, sensitizing them to inactivation by large molecules, including many interesting carcinpogens. This finding was a significant advance, contributing to the success of the diol-epoxide I experiments and holding promise for the future. 3) Lambda phage, a virus of Escherichia coli does not form many plaques on wild-type E. coli K if the DNA of the lambda is not modified by E. coli K specific

methylation. Using appropriate E. coli mutants, lambda phage lacking "K-specific" methylation was prepared. It was then shown that ultraviolet irradiation of the E. coli allows the normally restricted plaque formation to occur. Furthermore the effect occurs at lower ultraviolet doses if an E. coli mutant which is unable to repair pyrimidine dimers is irradiated, indicating that repair of ultraviolet damage is important in the phenomenon. This phenomenon is related to the mechanism by which E. coli is mutated by ultraviolet light. 4) A mutant of adenovirus 2 which grows on 8 strains of human tumor cells, but does not make plaques on normal human fibroblasts was partly characterized. DNA synthesis by the mutant virus is slower than that by wild-type adenovirus 2 when infecting normal human fibroblasts.

Significance to Biomedical Research and the Program of the Institute: Physical, chemical, and viral carcinogens are all known to alter the structural integrity of the cellular genetic apparatus. An evaluation of the role of DNA repair and/or related mechanisms in conferring resistance or susceptibility to mutagenesis and carcinogenesis is an important facet in any overall program having as its goal the understanding of the molecular pathways which, when perturbed, give rise to carcinogenesis in humans. It is the long range goal of this project to determine whether or not the elucidation of genetic repair mechanisms is important to the understanding of carcinogenesis, and it is expected that an understanding of genetic repair mechanisms in general will benefit many areas of biomedical research.

Proposed Course of Project: To pursue the goals outlined above in Objectives and to publish these results.

Publications

Lytle, C. D., Day, R. S., III, Hellman, K. B., and Bockstahler, L. E.: Infection of UV-irradiated xeroderma pigmentosum fibroblasts by Herpes simplex virus: Study of capacity and Weigle reactivation. Mutat. Res. 36: 257-264, 1976.

Day, R. S., III.: Caffeine inhibition of the repair of ultraviolet-irradiated adenovirus in human cells. Mutat. Res. 33: 321-326, 1975.

Day, R. S., III, Giuffrida, A. S., and Dingman, C. W.: Repair by human cells of adenovirus-2 damaged by psoralen plus near ultraviolet light treatment. Mutat. Res. 33: 31-319, 1975.

Day, R. S., III.: UV-induced alleviation of K-specific restriction of bacteriophage . J. Virol. (in press).

Day, R. S., III, and DiMattina, M.: Photodynamic action of chlorpromazine on adenovirus 5: Repairable damage and single-strand breaks. Chem. Biol. Interact. (in press).

SUMMARY REPORT

EXPERIMENTAL PATHOLOGY BRANCH

July 1, 1976 through September 30, 1977

"Plans, develops, and implements a coordinated research program involving (1) study of experimental tumor pathology by morphological and functional methods; (2) development and characterization of experimental models of major forms of human cancer using in vivo and in vitro carcinogenesis methods; (3) development of techniques for the experimental study of the pathogenesis of cancer in human tissues; (4) morphological and biochemical studies on chemical carcinogenesis and its inhibition in selected animal tissues; and (5) research on the etiology and pathogenesis of cancer related to perinatal exposures."

In July 1976 the Tumor Pathology Section was administratively reassigned from this Branch to the Carcinogenesis Bioassay Program.

The Branch now consists of the Office of the Chief and four Sections: "Differentiation Control", "Human Tissue Studies", "In Vitro Pathogenesis", and "Perinatal Carcinogenesis". Investigators in different sections of this Branch collaborate in their studies on mechanisms and models of carcinogenesis.

The main focus of investigations in the Branch is on the study of the pathogenesis of neoplastic disease using morphological and biochemical approaches at all levels of biological organization, including human tissues, animal models, organ and cell cultures as well as molecular interactions.

Particular emphasis is given to carcinogenesis studies with epithelial cells, using both animal and human tissue sources and in vitro culture methods as well as whole animal experiments.

There is a fundamental need to relate the process of carcinogenesis to the specific characteristics of the tissues from which the induced tumors originate. Chemical carcinogenesis is the result of a chemical-biological interaction resulting in a variety of pathologic responses characteristic of the tissues and cells of origin and of the conditions of exposure. Cancer in man is similarly characterized by a wide variety of pathologic response patterns.

The advancement of knowledge in carcinogenesis has made it possible, particularly in recent years, to develop numerous animal models for major forms of human cancer, especially those of epithelial origin. These models become tools for studying the pathogenesis of major types of cancer. Such studies have rapidly advanced by combining old and new research approaches and using both morphological and biochemical methods.

Most of the current knowledge of carcinogenesis comes from studies in animals, and a great deal of uncertainty still exists on how to correlate animal with human pathology. This is a crucial problem in the interpretation of research findings in terms of human cancer pathogenesis and also in the assessment of carcinogenic risks for humans on the basis of experimental animal studies.

An effective approach to this problem has been established by developing methods for the experimental study of the action of carcinogens directly on human target tissues. This was accomplished - ethically and effectively - using organ and cell cultures of human tissues, particularly from the epithelial organs in which major types of human cancers are induced such as bronchus, colon and pancreas.

Metabolism of several types of carcinogens, their localization and binding to cellular macromolecules have been identified for the first time in their human target tissues. The major adduct formed by the interaction of benzo[a]pyrene with DNA in human bronchus has been identified and found to be similar to that previously identified in the experimental animals. A wide interindividual variation was found in the level of carcinogen binding to tissues from different individuals and the significance of this parameter as an indicator of susceptibility is under investigation.

Mouse epidermal cell culture methods previously established in this Branch have been further developed for use in the study of the mechanisms of neoplastic transformation and of tumor promotion in these cells, for which there is a very extensive counterpart of in vivo studies on carcinogenesis and promotion. The action of direct carcinogens, promoting agents and inhibitors has been investigated at the morphological and biochemical level.

The interactions of carcinogens with mammalian DNA were studied in several systems, with emphasis on DNA repair mechanisms, identification of the adducts formed by carcinogens with DNA bases, and human-tissue-mediated activation of carcinogens as mutagens.

Further advances have been made in the study of specific organotropic effects of different carcinogens, on the role of age in the response to carcinogens, in the study of transplacental carcinogenesis and in the elucidation of the histopathogenesis of selected cancers.

Further work on the mode of action of vitamin A and derivatives in the control of epithelial differentiation and carcinogenesis has elucidated additional steps in this complex mechanism, particularly identifying certain structural requirements for the activity of retinol and derivatives in the biosynthesis of specific mannose-containing glycoproteins.

Office of the Branch Chief - The following contributions were made by staff members in this Office.

Definitions and classifications for toxic and carcinogenic effects were outlined. Criteria for the evaluation of environmental carcinogens were developed and scientific advice was provided to several agencies of the Federal Government and to other organizations, nationally and internationally.

A scientific conference and workshop on "In Vitro Carcinogenesis" was organized (July 1976) to provide an up-to-date review of new methods and studies in carcinogenesis on cells and tissues in culture. A resulting reference volume has been prepared for publication.

The histopathogenesis of adenocarcinoma of the pancreas, a major type of cancer (4th leading cause of cancer mortality in the USA) has been investigated using the recently developed nitrosamine-induced hamster model. This study suggests that this cancer arises from pancreatic duct epithelium, progressing from duct hyperplasia to cystadenomas, to intraductal and invasive ductal carcinomas.

Differentiation Control Section - *"(a) Studies molecular processes involved in the control of cell differentiation with emphasis on maintenance of the normal phenotype and induction of new phenotypic expression by dietary, hormonal carcinogenic compounds, in the whole animal and in cultured cells; (b) Investigates the biochemical mode of action whereby selected compounds inhibit or prevent the development of epithelial cancer; and (c) Studies qualitative and quantitative alterations in biosynthetic and structural properties of membrane systems from normal, transforming and transformed tissues in culture and in vivo."*

The mode of action of vitamin A in maintaining growth and epithelial differentiation has been elusive for some sixty years. The inhibition of epithelial carcinogenesis by vitamin A and related compounds has been identified in the last decade. Deficiency of this essential nutrient precipitates a cascade of biochemical events which appear to involve mostly epithelial tissues. To relate these events to the molecular mode of action of vitamin A has proven difficult. Considerable progress has been made recently with the finding that vitamin A controls the biosynthesis of specific glycoproteins.

In addition to previous results on the specific and drastic effect of vitamin A deficiency and excess on intestinal secretory glycoprotein synthesis, it was shown that the vitamin regulates the biosynthesis of liver membrane glycoproteins. Deficiency of this nutrient causes a 90% or greater decrease in the incorporation of [^{14}C]mannose into hamster liver glycolipids and glycoproteins. Conversely, high levels of retinylpalmitate cause a two to four-fold increase in such biosynthetic processes. In deficiency, the amount of covalently-bound mannose in different hamster liver mannoconjugates was reduced to 21% of normal levels, although liver weight was unaltered.

These findings suggested the hypothesis that the vitamin may be molecularly involved in the process of synthesis of mannoconjugates in mammalian membranes. Bacterial membrane mannoconjugates are assembled by a process which involves the transfer of mannose and other monosaccharides from the water soluble sugar nucleotide to a hydrophobic compound (polyprenyl phosphate) which resides in the hydrophobic environment of the membrane. Different polyprenylglycosylphosphates have been described which have the function of adding glycosyl units to the growing polysaccharide chains of glycoconjugates. Such glycosyl units dictate in some cases the antigenic structure and potential of the bacterial coat.

Retinylphosphate was found to be a necessary intermediate for the formation of glycosylretinyl phosphates in mammals. Its presence in intestinal epithelial cells was demonstrated in amounts of 0.035 µg/g of wet intestinal mucosa. Its full characterization was possible by comparative studies with chemically synthesized retinylphosphate.

Mannosylretinylphosphate was found to be the second intermediate. It was synthesized enzymatically from retinylphosphate and guanosinediphosphate mannose. Mannosylretinylphosphate was an effective donor of mannose to endogenous glycoprotein acceptors of the rat liver membrane. The anomeric configuration of the mannosyl residue is β. Characteristics of the acceptor glycoprotein(s) include its lability to mild alkali which suggests a glycoprotein containing an O-glycosidic linkage between the oligosaccharide chain and the protein core.

Such glycoprotein acceptor differs from glycoproteins which use dolichyl mannosylphosphate as the lipid intermediate: the latter family of glycoproteins contains N-glycosidically linked oligosaccharides. Thus, vitamin A appears to function as a carrier of mannose for specific glycoproteins of the membrane.

Structural modifications consented to the vitamin with retention of its function in mannosyl transfer were investigated. A variety of retinyl phosphate derivatives were shown to function as mannosyl acceptors, while their retinoic acid counterparts showed no activity. This finding suggests that metabolic alteration of retinoic acid may occur prior to its phosphorylation.

A study of the effect of chemical carcinogens on glycolipid, glycoprotein and glycosyl transferases of the surface membrane was initiated. Cultured human fibroblasts from the dermis of normal individuals and from individuals with basal cell nevus syndrome are being used for these studies.

Human Tissue Studies Section - *"(a) Conducts carcinogenesis studies on the pathogenesis of human cancers, especially carcinomas, using tissues from human target organs and from experimental animals, by organ culture and related methods and by correlation with clinical studies; and (b) conducts ultrastructural studies, advises, and assists Carcinogenesis Program staff on electron microscopic and quantitative autoradiography techniques."*

Studies of carcinogenesis can now be conducted directly in human target tissues. The maintenance of human tissues in a controlled experimental setting provides model systems for: 1) identifying environmental carcinogens and mutagens, their metabolic pathways in target tissues and host factors determining oncogenic susceptibility; and 2) developing new methods of prophylactic intervention in individuals at high risk of developing cancer. In parallel with the studies of human tissues, an appropriate animal model for each target site is being investigated. This approach should provide a link between carcinogenesis studies using experimental animals and carcinogenesis in humans.

Model systems have been developed for human bronchus and pancreatic duct and are currently being refined for human colon and breast. These model systems have three major facets: 1) collection of viable human tissues, 2) in vitro maintenance of the tissues, and 3) xenotransplantation of human tissues into immunodeficient animals to assess tumorigenicity of suspected malignant cells transformed in vitro. Human tissues can be exposed to carcinogens and to anticarcinogens while in culture and/or as a xenograft. Explant cultures of human tissues have the advantage of retaining normal cellular differentiation and

the 3-dimensional intercellular relationships. Monolayer epithelial cell cultures can be initiated from explants and used for carcinogenesis studies.

The in vitro maintenance of human bronchus and pancreatic duct in a controlled experimental setting provides an excellent system to study the metabolism of chemical carcinogens including those found in tobacco smoke and in the environment. Cultured human bronchi metabolically activate procarcinogens [polynuclear aromatic hydrocarbons: 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, benzo[a]pyrene (BP) and dibenz[a,h]anthracene; N-nitrosamines: dimethylnitrosamine (DMN), diethylnitrosamine (DEN), N-nitrosopiperidine (NPd), N-nitrosopyrrolidine (NPy) and dinitrosopiperazine (DNP); a hydrazine: 1,2-dimethylhydrazine (1,2-DMH); and a mycotoxin: aflatoxin B_1 (AFB_1)] into forms that bind to cellular macromolecules including DNA.

The pathway of BP metabolism leading to the binding (-)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrohydrobenzo[a]pyrene to DNA in cultured human bronchial mucosa has been defined. The major BP-DNA adduct is between the 10-position of BP and the 2-amino group of guanine. Therefore, the metabolic pathway forming the predominant BP-DNA adduct in human bronchus is similar to that found in experimental animals in which BP is carcinogenic.

An individual's risk of developing cancer may depend, in part, on the balance between activation and deactivation of procarcinogens. Initial studies have focused on the formation of BP-DNA adducts as a resultant of these competing metabolic processes. The binding of BP to DNA in cultured human bronchus was measured in specimens from 57 patients. A 75-fold variation in binding levels was observed which is similar in magnitude to that found in pharmacogenetic studies of drug metabolism. When compared to patients without lung cancer and to those with adenocarcinomas of the lung, nontumorous specimens from patients with epidermoid lung cancer has significantly higher levels of BP bound to DNA.

Metabolism of carcinogenic N-nitrosamines was studied in normal-appearing bronchial specimens obtained from four patients. Explants of bronchi were cultured in a chemically defined medium for seven days. N-Nitrosoamines [DMN, DEN, DNP, NPy, and NPd] labeled with ^{14}C were each added to the medium at a concentration of 100 μM for 24 hr. Measurable CO_2 was formed by bronchial explants from: 1) DMN, DEN and NPy in all four patients; 2) DNP in three of four patients; and 3) NPd in only one of four patients. In all bronchial specimens, binding of these N-nitrosamines and/or their metabolites to bronchial mucosal DNA and protein was found. Binding levels were higher to protein than to DNA. DMN methylated DNA at both O-6 and N-7 positions of guanine. Binding levels of DNP were as high as those with the two acyclic N-nitrosamines, DMN and DEN, while binding levels of MPy and NPd were lower. Human bronchus can metabolize acyclic and cyclic N-nitrosamines that are found in the environment and in tobacco smoke.

Both 1,2-DMH and AFB_1 are metabolized by cultured human bronchi. Binding of these carcinogens to macromolecules was dependent on dose. 1,2-DMH methylated guanine in DNA at both O-6 and N-7 positions. The AFB_1-DNA adduct(s) has not been identified.

The metabolism of BP by human bronchus and by pulmonary alveolar macrophages (PAM) from the same donor was studied to investigate whether the PAM might be useful as an indicator cell for the human bronchus. PAM enzymatically convert BP into metabolites that bind to cellular macromolecules. A 9-fold inter-individual variation was found in the binding to DNA. The majority of the metabolites of BP were water-soluble, while 7,8-dihydro-7,8-dihydroxy BP (7,8-diol) 9,10-dihydro-9-10-dihydroxy BP (9,10-diol), triols and tetrols were the major metabolites in the fraction extractable by ethyl acetate. PAM metabolize BP into its proximate carcinogenic form, 7,8-diol, which is then released into the extracellular space. While PAM may play an important role in metabolism of inhaled carcinogens, preliminary data suggest that PAM will not be useful as an indicator cell for human bronchus; no correlation of AHH activity and binding to DNA or protein between PAM and bronchus was found.

Explant cultures of both human and rat colon have the ability to enzymatically convert several classes of chemical carcinogens, such as polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins, and hydrazines, into metabolites which react with cellular macromolecules. The effect of various co- and anti-carcinogens of colon carcinogenesis in rats on the metabolism of BP and 1,2-DMH by cultured rat colon has been studied. Different segments of the rat colon metabolize BP and 1,2-DMH to varying extents. The metabolism of BP in the colon is approximately 10% of that found in the human bronchus; the major metabolites, extractable with ethyl acetate, were quinones, a peak containing 9,10-diol and 7/8,9-triol and 7,8-diol. The metabolite patterns of BP in human and rat colon were quite similar. Secondary bile acids significantly described the binding of 1,2-DMH to rat colon, while enhancing the binding level of BP to DNA in human colon. Benz[a]anthracene did not enhance activity of AHH or BP binding to DNA in either human or rat colon.

Since most chemical carcinogens are also mutagens, assays of mutagenesis are useful as screening tests for chemical carcinogens. In these assays, promutagens (procarcinogens) are activated by microsomes from either experimental animals or from human tissues. However, metabolic activation of procarcinogens by microsomal preparations may not reflect metabolic activation within intact cells. A mutagenesis assay has now been developed in which promutagens are metabolically activated by explants of human target tissues, e.g., bronchus. Mutation frequency (resistance to either 8-azaguanine or ouabain) is measured in a mammalian cell line, V-79 Chinese hamster cells, which does not metabolically activate polynuclear aromatic hydrocarbons into mutagens. When human bronchial explants are cocultivated with V-79 cells in medium containing either BP or its proximate carcinogen, 7,8-diol, a marked increase in mutation frequency is observed. This increase is dependent upon dose of either BP or 7,8-diol, duration of cocultivation, and amount of human bronchus. At equimolar concentrations, 7,8-diol was activated to at least a 5-fold more potent mutagen than its parent compound, BP. Human tissue mediated mutagenesis tests have potential for: 1) screening tests for chemical carcinogens; and 2) biological assays to be used with biochemical assays in an effort to determine oncogenic susceptibility among individuals as well as among different target tissues in a single individual.

An international workshop entitled "Carcinogenesis Studies in Human Tissues and Cells" has been organized by this Section. The program covers many facets of this area of research including methods to isolate and culture human

epithelial cells, explant culture and xenotransplantation of human tissues, metabolism of carcinogens by human tissues and cells and, finally, mutagenesis and malignant transformation of human cells. In addition to formal presentations, "state of the art" group discussions and laboratory sessions are important features of the program.

In conclusion, experimental models using human tissues and cells are providing a needed link between carcinogenesis in human and investigations with experimental animals.

In Vitro Pathogenesis Section - *"(1) In collaboration with other Sections of the Branch, develops in vitro models for studying the pathogenesis of major forms of human cancer utilizing both human and animal models; (2) utilizes these culture systems in biochemical, cytologic, and biologic investigations to develop approaches to the prevention of cancer in humans, specifically by studying the stages in the progression of the disease before it threatens life; and (3) develops procedures for evaluating the biological potentiality of lesions induced by chemical or physical carcinogens in mammalian tissues in vitro."*

This Section has directed its efforts towards developing in vitro model systems to study mechanisms of epithelial carcinogenesis, since the large majority of human cancers are derived from epithelial cells. A classic model for induction of squamous cancer, mouse epidermis, has been adapted for growth in cell culture in this laboratory and previously shown to maintain biological function in a reasonably normal manner. During the past year additional studies were completed which further defined the epidermal in vitro model. Experiments on serum and medium effects revealed that 10% serum is optimum for overall growth at 37^{o} but proliferation and differentiation are accelerated in 2% serum. Low temperature (31^{o}) and low pH (6.7) inhibited growth and differentiation while pH 7.6 accelerated these parameters. The use of fibroblast coated substrate, either irradiated or ethanol fixed, enhanced plating efficiency and prolonged the ability to subculture by about 2 fold over plastic substrates for skin from Balb/c mice but such substrates were not necessary for prolonged in vitro growth and survival when mice from a skin tumor sensitive strain (SENCAR) were donors. Spontaneous conversion to long-term growth occurred with a low frequency in Balb/c cells and a high frequency in SENCAR cells but in the absence of carcinogen treatment such lines are not tumorigenic. After treatment with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a potent skin carcinogen, both Balb/c and SENCAR cells convert to long-term growth with a higher frequency and some of these lines are tumorigenic. These transformed epidermal cells show an excellent correlation between the ability to produce tumors in animals and ability to grow in soft agar in vitro. There is also a good correlation between tumorigenicity and production and secretion of plasminogen activator. Immunological markers for differentiation and transformation are also being generated using these lines. Antibodies to cell surface antigens of normal and transformed cells and to keratin proteins synthesized in vitro have been produced in rabbits. Human epidermal cells grow and differentiate exceptionally well in vitro and should be useful to study mechanisms of carcinogenesis in human tissues.

Studies on the interaction of carcinogens with the mammalian genome have been performed utilizing mouse epidermal cell cultures. Both excision repair and post-replication repair occur after treatment with MNNG or ultraviolet light. SENCAR mice appear to have a reduced excision repair capacity. Tumor promoters inhibit repair at early times after exposure (within 24 hours) but are without effect at later times or when given after the damaging agent. An antibody has been prepared against the guanosine (G) adduct of the carcinogen N-acetoxy-acetylaminofluorene (NA-AAF). This antibody is specific for G-AAF and when used in a radioimmunoassay is sensitive enough to detect 0.5-1 nanograms of G-AAF. This assay is now being utilized to detect adduct formation in DNA from cells treated with NA-AAF and to follow its repair.

Mouse epidermal cell cultures appear to be an excellent model for assay and mechanistic studies of tumor promotion, a potentially reversible phenomenon. Brief exposures to small doses of phorbol esters stimulate ornithine decarboxylase activity 10-15 fold and DNA synthesis 5-15 fold. Both of these effects occur in vivo. These responses are dose dependent, may be cell cycle specific, and vary directly with the promoting potency of a series of phorbol esters. The ODC assay can detect several classes of promoting substances including phorbol esters, anthralin, polychlorinated biphenyls and tweens. Polyamines and particularly putrescine appear to be a mediator of the phorbol ester promoting response in vitro. Exogenous putrescine enhances and prolongs the promoter induced stimulation of proliferation. The anti-inflammatory and anti-promoting steroid fluocinolone acetonide (FA) inhibits promoter induced growth stimulation in vivo and in vitro. In culture the effect is most pronounced when FA is given prior to promoter and its presence after treatment is not required. Paradoxically, FA treatment potentiates the ODC response to promoters both in vivo and in vitro. In culture the growth inhibitory activity of FA can be overcome by addition of exogenous putrescine to the medium. These results suggest that steroids act by interfering with the cellular responses to endogenous polyamines.

Epidermal cells are useful for studying differentiation also. Keratin proteins are synthesized in vitro and slough into the medium. Polyacrylamide gel electrophoreses of such products are identical to those of stratum corneum. This process can be altered by low temperature growth, treatment with tumor promoters, or retinyl acetate (RA). This latter treatment causes remarkable changes in incorporation of sugars into glycoproteins within 1 hour of RA exposure. These changes appear to be mediated through the production of retinyl-phosphate-sugar intermediates.

The Section also contributes, through the collaborative program, to studies for other in vitro models of epithelial carcinogenesis respresenting major forms of human cancer: projects developing cell and organ cultures of pancreatic and prostatic epithelium and liver are in progress. In addition, several studies to identify in vitro markers for recognizing malignant transformation in epithelial cells are being developed.

Perinatal Carcinogenesis Section - *"(1) Investigates the induction of cancer in experimental animals before birth and during infancy, with the goal of identifying factors responsible for the initiation and growth of tumors of childhood and infancy in man; (2) utilizes techniques of chemical*

synthesis, biochemistry, histopathology, immunology, and endocrinology to identify carcinogens to which both individually and in combination the fetus and neonate are particularly vulnerable, and to identify host factors which qualitatively modify their biological effects; and (3) develops measures for prevention of cancer in children or in later life as a response to conditions of high susceptibility to carcinogens during fetal life or childhood."

Studies have continued on the carcinogenic effects of synthetic α-hydroxy derivatives of dimethylnitrosamine (DMN) as models of the postulated active metabolite of DMN, methyl(hydroxymethyl)nitrosamine. Methyl(acetoxymethyl)-nitrosamine (DMN-OAc) was previously reported to be highly carcinogenic and absolutely selective for the mucosa of the small intestine and colon when given as a single IP injection to weanling rats. Further studies have demonstrated that the organotropism of DMN-OAc is dependent on the route of injection, and that tumors of the intestines are rarely induced when the carcinogen is administered to rats by routes other than IP. This instance of organotropism is apparently pharmacodynamic, rather than metabolic, in origin. Non-mucinous adenocarcinomas of the intestines induced by single IP injections of DMN OAc grow large, but rarely metastasize, in contrast to the behavior of similar neoplasms induced by multiple doses of other agents. The possible dose-dependence of metastatic tendency is under continuing investigation.

Collaborative studies on transplacental carcinogenesis in Erythrocebus patas, a non-human primate, have continued in collaboration with Drs. W. London and A. Palmer, NINCDS. Repeated IV administration of ethylnitrosourea (ENU) to juvenile monkeys has resulted in a high incidence of tumors during the third year after initiation of treatment. Most tumors were mesenchymal origin, arising chiefly from bone, lymphoreticular, or blood vascular tissues. Transplacental exposure to ENU begun during the first month of pregnancy has resulted in tumor development during the first year of prenatal life in 4 animals. One tumor was present at birth. Monkeys which received comparable transplacental doses of ENU, but begun later in gestation, have developed no tumors postnatally after as long as 3 years of observation. These observations comprise the first experimental demonstration of transplacental carcinogenesis in a primate species, and indicate that the period of maximal susceptibility to direct-acting transplacental carcinogens is much earlier in gestation in this species than had been predicted from studies in rodents.

Transplantation immunity to certain transplacentally induced lung tumors in mice has previously been shown to be due principally to an antigen common to several C3Hf, DBA/2, and C57BL/6 tumors and also found in normal lung tissue of lung tumor-susceptible strain A ($H\text{-}2^a$), but not in normal tissues of strains C3Hf ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$), or DBA/2 ($H\text{-}2^d$). Genetic control of expression of this antigen has now been shown to behave as a single autosomal dominant trait, closely linked to the K end of the $H\text{-}2^a$ allele of the major histocompatibility complex. The potential exploitability of this antigenic system for immunoprophylaxis of transplacentally induced lung tumors is under investigation.

New projects have been initiated on the roles of DNA repair in the enhanced susceptibility of certain fetal tissues to carcinogens, and the fidelity of repair of lesions inflicted in DNA by different classes of carcinogens and

mutagens. Another new project explores the role of differentiation in mouse and rat metanephric tissue as a determinant of the histological patterns and biologic behavior of the different types of renal tumors inducible transplacentally in these species.

Tumor Pathology Section - *"Plans, develops and implements a program to: 1) provides diagnostic pathology criteria, standardization and monitoring for the Carcinogenesis Bioassay Testing Program and related criteria for the development of experimental tumor models; 2) provides guidance consultation and training in tumor pathology for both program and contract staff as well as consultation services to other national and international agencies."*

In July 1976 the Tumor Pathology Section was administratively reassigned from this Branch to the Carcinogenesis Bioassay Program.

There have been a number of changes in both responsibilities of the section and in personnel during the past year. Dr. Robert A. Squire became Acting Director of the Carcinogenesis Bioassay Testing Program. Dr. Morton H. Levitt was converted from a staff fellow to a permanent PHS officer and moved to the Experimental Pathology Branch. Dr. Jerrold Ward, a veterinary pathologist with interest and expertise in laboratory animal pathology and experimental carcinogenesis has joined the staff. In addition, several pathologists are being recruited.

A much greater proportion of time has been spent in providing pathology expertise to the Carcinogenesis Bioassay Testing Program including providing pathology monitoring and pathology criteria. A major activity again this year was the verification of pathology end-points and review and editing of pathology narratives on tests which were completed in the bioassay program. In order to deal with the number of compounds being reviewed, several working teams comprised of pathologists from NCI and from the NCI pathology support contractors have been formed. In addition, summary reports of the experiments including pathology results were reviewed by the section pathologists.

Last year the tumor nomenclature of the Systematized Nomemclature of Pathology (SNOP) was modified by the Tumor Pathology Section with the assistance of several pathologists from the collaborative bioassay program for coding tumor diagnoses in rats and mice into the Carcinogenesis Bioassay Data System. This year, this work was extended to include the non-tumor diagnoses resulting in a modified morphology section of SNOP for use in the Bioassay Program. In addition, the modified SNOP has been alphabetized for greater ease in using it. Use of this document has already decreased the amount of editing and verification required and should have an even greater impact in the future. Plans are being made to modify the topograph section when time and resources permit.

Research activities in the Section include a study on the effects of irradiation on the spontaneous tumors in mastomys. However no results are available at this time.

In collaboration with the Lung Cancer Branch, several studies investigated the possible protective effects of various synthetic retinoids on tumors induced in several systems by different chemical carcinogens. These include NMU-induced

bladder cancer, hydroxy-BBN-induced bladder tumors, NMU-induced mammary tumors and NMU-induced colon cancer all in rats. The study, begun last year, ultilizing the NMU-induced bladder cancer system has been completed and 12-cis-retinoic acid was found to have a significant inhibitory effect on the development of epithelial bladder tumors. A similar effect with retinyl acetate was found in the NMU-induced mammary tumor system. The other 2 studies are still being evaluated.

Weekly slide conferences continue to be held and attendance and participation have increased. These sessions allow presentation and discussion of interesting lesions from a variety of studies. These sessions have been expanded to include a short oral presentation once a month by various attendees.

The section continues to supply pathology expertise for many aspects of the Carcinogenesis Program on a consultative basis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP-04482-01 EXP

PERIOD COVERED
May 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogenicity of 2,6-dimethylnitrosomorpholine (2,6-DMNM) in the Syrian Hamster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Levitt	Research Pathologist	EXP, NCI
OTHER:	M. S. Sporn	Chief, Lung Cancer Branch	EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to conduct preliminary tumorigenicity studies with 2,6-DMNM in the Syrian golden hamster to confirm the effectiveness of this model for a subsequent chronic study to evaluate the role of 13-cis-retinoic acid and related synthetic vitamin A derivatives on possible inhibition of hamster pancreatic carcinogenesis. The preliminary study will assess (1) the effectiveness of 2,6-DMNM in the Syrian hamster as a specific pancreatic carcinogen; (2) the number of induced neoplasms, if any, in organ systems other than the pancreas; (3) the effectiveness of the agent by both subcutaneous and intragastric routes of administration; and (4) histopathologic evaluation of the liver in terms of lethal or sublethal cellular injury (which must be kept at a minimum if a vitamin A analogue is to be successful in this model system).

PHS-6040
(Rev. 10-76)

Objectives: To determine the effectiveness of 2,6-dimethylnitrosomorpholine (2,6-DMNM) as a specific pancreatic carcinogen in the Syrian golden hamster preliminary to a chronic study to evaluate the role of 13-cis-retinoic acid and related synthetic vitamin A derivatives on the possible inhibition of pancreatic carcinogenesis.

Methods Employed: Three hundred forty outbred male Syrian golden hamsters, weighing 80-100 grams at the start, will receive treatments according to the following:

Group[a] No.	No. of Doses	Each Dose	Route	Total Dose 2,6-DMNM	Dietary[b] Supplement
1	10(1x/week,10 weeks)	160mg/kg	IG	1600	Gelatin only
2	"	160mg/kg	IG	1600	CRA [c,d]
3	"	160mg/kg	SC	1600	Gelatin only
4	"	160mg/kg	SC	1600	CRA
5	"	80mg/kg	IG	800	Gelatin only
6	"	80mg/kg	IG	800	CRA [c,d]
7	"	80mg/kg	SC	800	Gelatin only
8	"	80mg/kg	SC	800	CRA
9	"	40mg/kg	IG	400	Gelatin only
10	"	40mg/kg	IG	400	CRA [c,d]
11	"	40mg/kg	SC	400	Gelatin only
12	"	40mg/kg	SC	400	CRA
13	"	0.2ml oil	IG	0	Gelatin only
14	"	0.2ml oil	IG	0	CRA
15	"	0.2ml oil	SC	0	Gelatin only
16	"	0.2ml oil	SC	0	CRA
17 (shelf control)	-	-	-	-	-

[a] Carcinogen to be administrated in 0.2 ml olive oil. Sacrifice animals at 15,25,40 weeks (6 animals each time). Hamsters are to be 8 weeks old male Syrian Golden at start of experiment.

[b] Basal ratio Purina Lab Chow in meal form

[c] CRA = 13-cis-retinoic acid (140mg/kg diet) in gelatin beadlets

[d] Replace dietary supplement with basal rations 24 hours before DMNM administration until 24 hours after each dose.

Animals will then be examined by complete gross necropsy and routine light microscopy (to include all gross lesions, tissue masses or suspect tumors and

regional lymph nodes, trachea/larynx, lungs/main stem bronchi, nasal cavity, liver, pancreas, spleen, and kidneys).

Major Findings: This is a new project and results are expected by late 1977.

Significance to Biomedical Research and the Program of the Institute: Pancreatic cancer is now the 4th leading cause of cancer mortality in the United States, with an incidence that is rising alarmingly. Since the introduction of the hamster model for pancreatic carcinogenesis by Pour et al. (Am. J. Pathol. 76: 349, 1974), demonstrating a high incidence of induced pancreatic neoplasms with N-nitroso-bis(2-hydroxypropyl)amine (BHP), considerable work on the metabolism of BHP and related symmetric di-alkylnitrosamines has suggested that N-nitroso-(2-hydroxypropyl) (2-oxopropyl)amine (HPOP) is a major metabolite (and possibly the proximate carcinogen) of the potent pancreatic carcinogen N-nitroso-bis(2-oxopropyl)amine (BOP), which is, of course, closely related to BHP. Moreover, HPOP and BHP are also putative metabolites of 2,6-DMNM in hamsters. Structurally, 2,6-DMNM and the cyclic acetal form of HPOP differ only in the presence of a hydroxy group at the 2-position in the latter compound. Preliminary investigations with 2,6-DMNM by Mohr et al. (J. Natl. Cancer Inst. 58: 429, 1977) have shown that when it is administered intragastrically to Syrian hamsters, over 70% of the animals developed pancreatic neoplasms, results also noteworthy for the significantly reduced incidence of tumors at other sites such as the respiratory tract and liver.

Proposed Course of the Project: Important studies confirming the inhibitory effect of the retinoids on tumorigenesis in several animal model systems including bladder, colon, esophagus, breast and lung have suggested a very important role for these vitamin A analogues as tumor inhibitors. The possibility of extending this work to the pancreas is the intent of this project.

Publications

None, as this is a new project.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP-04493-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Explant Culture of Human Bronchus and Peripheral Lung.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Stoner	Research Scientist	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
	H. Autrup	Staff Fellow	EXP, NCI
	B. Trump	Chairman of Pathology	Univ. of MD
	M. Levitt	Research Pathologist	EXP, NCI
OTHER:	E. McDowell	Associate Professor of Pathology	Univ. of MD
	L. Barrett	Instructor, Dept. of Pathology	Univ. of MD
	E. Kingsbury	Princ. Invest. Litton Bionetics	Kensington, MD

COOPERATING UNITS (if any)
University of Maryland, School of Medicine, Baltimore, MD
Litton-Bionetics Inc., Kensington, MD

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Model systems for the study of carcinogenesis in cultured human bronchus and peripheral lung are being developed. Explants of human bronchus and peripheral lung have been maintained in culture for periods of 3 months and 25 days respectively. The metabolism of chemical carcinogens is being investigated in these tissues. Attempts are being made to culture normal human bronchial epithelial cells in a feeder layer of X-irradiated human lung fibroblasts. It is anticipated that these model systems will be useful for; 1) Identification of environmental carcinogens for bronchus and peripheral lung; 2) Determination of the metabolic pathways for carcinogens in these tissues; 3) Identifying host factors determining susceptibility to chemically induced lung cancer; and 4) Evaluating new methods of prophylactic intervention in populations at high risk of developing lung cancer.

PHS-6040
(Rev. 10-76)

Objectives: To develop model systems to study chemical carcinogenesis in human lung tissues. These systems involve 2 major facets: 1) Obtainment of viable human lung; and 2) Maintenance of epithelial cell types in lung tissue in explant and cell cultures.

Methods Employed: Human lung tissues are obtained at either surgery or "immediate" autopsy. The explants are maintained in an organ culture chamber under controlled environmental conditions. The viability of the explants is assessed by: 1) high resolution light microscopy and electron microscopy, and 2) radioautographic techniques using labelled precursors for DNA, RNA and protein. The metabolism of diverse chemical carcinogens; e.g., polycyclic hydrocarbons and nitrosamines, is being investigated in human bronchial explants. Studies are underway to establish cultures of normal human bronchial epithelial cells that can eventually be used for in vitro carcinogenesis studies. During culture of the bronchial explants, cell with an epithelial morphology migrate from the outer edge of the explant onto the culture dish. Attempts are being made to identify the cells by; (a) electron microscopy, and (b) immunofluorescent techniques using antibody prepared to cells obtained from fresh bronchial tissue, and to A, B, O blood group antigens. To obtain "pure" cultures of bronchial epithelial cells, the cells are being cloned in culture dishes containing X-irradiated human lung fibroblasts.

Major Findings: Human bronchi can be cultured in vitro for at least 4 weeks in chemically defined CMRL-1066 medium supplemented with insulin (1.0 µg/ml), hydrocortisone hemisuccinate (0.1 µg/ml), beta-retinyl acetate (0.1 µg/ml), 2 mM L-glutamine, penicillin G (100 units/ml), and streptomycin (100 µg/ml), and for at least 12 weeks in medium supplemented with 5% heat-inactivated fetal calf serum. During the first 4 weeks in serum-supplemented medium, the bronchial epithelial cells retain essentially normal morphology. They actively incorporate precursors into DNA, RNA and protein. After culture for more than eight weeks, the epithelium becomes progressively more hyperplastic and is composed principally of small mucous granule cells and basal cells. Mucous production decreases with increasing time of explant incubation.

During culture, bronchial cells migrate from the outer edge of the explants onto the culture dish. When grown in plastic tissue culture dishes in the above-described medium supplemented with 10% fetal calf serum, the cells can be subcultured 5-7 times. The population doubling time (5-10 days) estimated at subculture increases progressively with serial transfer. The plating efficiency when the cells are grown in regular or "conditioned" medium is 0%; i.e., no colonies form when 100 mm dishes are inoculated with as many as 2×10^3 cells per dish. Electron microscopy showed that the cells become rapidly undifferentiated when cultured under these conditions; cells examined at the third subculture did not contain mucous granules, cilia, desmosomes or other morphological features of bronchial epithelial cells. Recent data suggest that it may be possible to selectively isolate bronchial epithelial cells free of contaminating fibroblasts by placing the explants on dense feeder layers of x-irradiated human lung fibroblasts. After selective isolation, the epithelial cells can be grown when subcultured on feeder layers of lesser density. Studies are underway to establish the optimum culture

conditions for the growth of the cells, and for their identification during serial passage in vitro.

Explants of human peripheral lung tissues have been maintained in culture for a period of 25 days. During the incubation there was active incorporation of ^{3}H-thymidine into the nuclei of alveolar type II epithelial cells and bronchiolar epithelial cells. After 7-10 days, type II cells were the most predominant alveolar epithelial cell type. Studies with the electron microscope provided morphological evidence for the synthesis of lung surfactant and collagen in the explanted tissue. Therefore, two potential target cells in the peripheral lung cells have been maintained for prolonged periods in explant culture. Studies are currently underway to investigate the metabolism of polynuclear aromatic hydrocarbons in peripheral lung tissues.

Significance to Biomedical Research and to the Program of the Institute: The extrapolation of experimental animal data to man is a major problem in carcinogenesis. One approach to provide a link between these experimental data and human cancer is to develop model systems in cultured human tissues for carcinogenesis investigations. Such systems could be used for the identification of carcinogens and their metabolic pathways and ultimately, in the identification of individuals who are highly susceptible to chemical carcinogens.

Current results indicate that explants of human bronchus and peripheral lung can be maintained for prolonged periods in culture. The metabolism of chemical carcinogens can be investigated in these tissues as well as studies of carcinogen-mediated cell transformation. Monolayer cultures of bronchial epithelial cells can also be used for carcinogenesis studies and should permit large scale investigations of a variety of environmental chemicals.

Proposed Course of the Project: Attempts are being made to improve each facet of this program. Conditions for organ culture of human bronchi are being modified to prevent the hyperplasia associated with prolonged culture. Studies have been initiated to identify the epithelial cells in culture. The methods include: (a) Electron microscopy, and (b) The detection by immunofluorescence, of epithelial cell surface membrane antigens and products of epithelial cell synthesis; e.g., type IV collagen. In addition, research is underway to determine the best method for transferring the cells; i.e., by use of enzymes or mechanical means, and to define the optimum growth conditions for the cells; i.e., substrate, nutritional and atmospheric conditions. The methodologies developed for growing bronchial epithelial cells in vitro might also be applicable to the culture of other important human target epithelial cells; e.g., pancreatic duct cells and colon cells.

Publications

Barrett, L., McDowell, E., Harris, C., and Trump, B.: Studies on ischemic cell injury: X. Reversal of ischemic cell injury in hamster trachea and human bronchus by organ culture. Beit. Pathol., 1977, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP-04434-01 EXP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of the Covalent and Non-covalent Interactions Between Chemical Carcinogens and Components of the Cell Surface.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Luigi M. De Luca	Research Chemist	EXP, NCI
	Sergio Adamo	Visiting Fellow	EXP, NCI
OTHER:	P. V. Bhat	Visiting Fellow	EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Experimental Pathology Branch, Carcinogenesis Program

SECTION

Differentiation Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.7	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a new project which has the long term objective of identifying specific components of the cell surface which may interact with different families of chemical carcinogens and the consequences of such interactions on the metabolism of the cell.

PHS-6040
(Rev. 10-76)

Methods Employed: Cultured human cells will be treated with radioactively-labelled chemical carcinogens and components of the cell surface will be isolated to study covalent or non-covalent interactions.

Significance to Biomedical Research and the Program of the Institute: This project was conceived in view of recent findings which implicate cell-surface glycosyl transferases, glycoproteins and glycolipids in the regulation of expression of the social behaviour of cells. Severe modification of these molecules have been shown to be associated with chemical and viral transformation. Thus it is expected that this project will contribute to the understanding of the basis for modified behaviour of transforming and transformed cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04485-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mutagenesis Mediated By Human Tissues and Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Hsu	Research Scientist	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
OTHER:	G. Stoner	Research Scientist	EXP, NCI
	H. Autrup	Staff Fellow	EXP, NCI
	B. Trump	Chairman of Pathology	Univ. MD

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	0.75	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since most chemical carcinogens are also mutagens, assays of mutagenesis are useful as screening test for chemical carcinogens. A mutagenesis assay has now been developed in which promutagens (procarcinogens) are metabollically activated by explants of human target tissues, e.g., bronchus. Mutation frequency (resistance to either 8-azaguanine or ouabain) is measured in mammalian cell line, V-79 Chinese hamster cells, which do not metabolically activate polynuclear aromatic hydrocarbons into mutagens. When human bronchial explants are cocultivated with V-79 cells in medium containing either benzo[a]pyrene (BP) or its proximate carcinogen, 7,8-diol, a marked increase in mutation frequency is observed. This increase is dependent upon dose of either BP or 7,8-diol, duration of cocultivation, and amount of human bronchus. At equimolar concentrations, 7,8-diol was activated to at least a 5-fold more potent mutagen than its parent compound BP. This human tissue-mediated mutagenesis test has potential as a: 1) screening test for chemical carcinogens; and 2) biological assay to be used with biochemical assays in an effort to determine oncogenic susceptibility among individuals as well as among different target tissues in a single individual.

733

PHS-6040
(Rev. 10-76)

Objectives: To study the mutation of V-79 cells mediated by human tissues and determine the relation among mutagenicity by the tissue and the susceptibility of inidividual to cancer.

Methods Employed: Explant cultures of human tissues; cocultivation of V-79 cells (a Chinese hamster cell line) and human tissues in medium containing a suspected chemical carcinogen; selection of V-79 cell mutant by media containing either ouabain or 8-azaguanine; Hoechst-Giemsa chromosome stain to determine sister chromatid exchanges; isolation of carcinogen-DNA adducts in human tissues.

Major Findings: This is a new project. V-79 cells and explants of human bronchus were cocultivated in the media containing either benzo[a]pyrene (BP) or its 7,8-diol. Mutants of the treated V-79 cells were selected by subsequently growing the cells in media containing either ouabain or 8-azaquanine for 12-15 days. Mutations in V-79 cells are not induced by carcinogenic polynuclear hydrocarbons unless the V-79 cells are cocultivated with explants of human bronchus. The mutation frequency was proportional to the number of explants added, duration of cocultivation, and the extent of BP metabolism in explants from individual patients. When compared to BP, 7,8-diol was 5-10 times more potent as a mutagen in this human tissue mediated system.

Significance to Biomedical Research and The Program of the Institute: Most studies on chemical carcinogens were done in mice and in rats. Extrapolation of the results to humans is a complex process in part because of the differences in the metabolism of chemicals between species. The mutagenesis test described above will utilize human tissue from several different organs and individuals, and may provide clues in the search for host factors that influence oncogenic susceptibility.

Proposed Course of the Project: Continuation of the mutation studies mediated by human tissues and cells. Measurement of sister chromatid exchanges and DNA repair synthesis in V-79 cells in addition to mutation in these cells mediated by human tissues. To develop a human tissue-mediated microbial mutagenesis test which uses Salmonella as the indicator organism.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CP 04486-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Explant and Cell Culture of Human and Bovine Pancreatic Duct

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Stoner	Research Scientist	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
	B. Trump	Chairman of Pathology	University of MD
	M. Levitt	Research Pathologist	EXP, NCI
OTHER:	R. Jones	Assistant Professor of Pathology	University of MD
	M. Valerio	Princ. Invest., Litton Bionetics	Kensington, MD
	H. Autrup	Staff Fellow	EXP, NCI

COOPERATING UNITS (if any)
University of Maryland, School of Medicine, Baltimore, MD
Litton-Bionetics, Inc., Kensington, MD

LAB/BRANCH Experimental Pathology Branch, Carcinogenesis Program

SECTION Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Model systems for the study of carcinogenesis in cultured human and bovine pancreatic duct tissues are being developed. Explants of human and bovine pancreatic duct have been maintained in culture for periods of 60 and 85 days respectively. The metabolism of polycyclic aromatic hydrocarbons is being investigated in cultured human pancreatic duct. Cells with an epithelial morphology have been cultured from the bovine pancreatic duct and attempts are being made to transform the cells with chemical carcinogens. It is anticipated that these model systems will be useful for; 1) Identification of environmental carcinogens for the pancreatic duct; 2) Determination of the metabolic pathways for carcinogens in the pancreatic duct; 3) Identifying host factors determining suseptibility to chemically induced pancreatic cancer; and 4) Evaluating new methods of prophylactic intervention in populations at high risk of developing pancreatic cancer.

PHS-6040
(Rev. 10-76)

Objectives: To develop experimental systems to study carcinogenesis in human and bovine pancreatic duct. Such systems involve 3 major facets: 1) Obtaining of viable human and bovine ductal tissues; 2) In vitro maintenance of epithelial cells in these tissues in cell and organ culture; and 3) Xenotransplantation of human and bovine pancreatic duct into im unodeficient animals.

Methods Employed: Human pancreatic ductal tissues are obtained at either surgery or "immediate" autopsy. Bovine pancreatic tissues are obtained from a local abattoir. The tissue is maintained in culture as explants, isolated cells, or xenotransplanted into athymic nude mice. The tissues are exposed to chemical carcinogens in vitro and/or as a xenograft. The morphological lesions induced in explants or in xenotransplants are examined by light and electron microscopy. The cultured cells are assayed for carcinogen-induced transformation by determining their ability to form colonies in soft agar and to produce tumors after transplantation into athymic, nude mice. Binding of carcinogens to macromolecules is measured by biochemical and autoradiographic methods.

Major Findings: Normal human and bovine pancreatic ductal tissues have been maintained in explant culture for periods of 60 and 85 days respectively. The tissues are cultured in CMRL-1066 medium supplemented with 5% heat-inactivated fetal calf serum, insulin (1 µg/ml), hydrocortisone (0.1 µg/ml), beta-retinyl acetate (0.1 µg/ ml), penicillin (100 µ/ml) and streptomycin (100 µg/ml). The explants maintained good structural preservation during these periods and incorporated radioactive precursors into DNA, RNA and protein. During the culture, cells with an epithelial morphology migrate from the outer edge of the explant onto the culture dish. With the human ducts, the cells migrate from the explant but eventually stop growing before the culture becomes confluent. The cells at the edge of the outgrowth become multinucleat and accumulate lipid. Attempts to improve cell outgrowth by utilizing medium containing homologous serum (human placental serum) or a mixture of homologous serum and fetal calf serum have been unsuccessful. One attempt was made to grow the cells on an irradiated feeder layer of fibroblasts however, unlike human bronchial cells, the pancreatic cells did not migrate from the explant onto the feeder layer. It should be mentioned that principally due to the inability obtain large amounts of human pancreatic ductal tissue, the studies to date have been very limited in scope.

Cells with an epithelial morphology have been isolated from bovine pancreatic duct and propagated continuously in culture for 26 subcultures (80 doublings of the population). Eleven clones were isolated from the parent culture and, under the electron microscope, several clones possess characteristic features of duct cells; i.e., cells of some clones are of the "dark" type and possess an electron-dense nucleus and cytoplasm, rough endoplasmic reticulum and numerous mitochondria, and cells of other clones are the "light" type with an electron-lucent nucleus and cytoplasm, few cytoplasmic organelles, and microfilaments. Mucous granules have not been observed in any of the cultures. Immunofluorescent techniques are being used for further identification of the cells. Antisera is being prepared to cells of two representative clones for reaction with in vivo pancreatic ductal tissue, and antisera prepared against

in vivo pancreatic duct will be reacted with the cells in culture. Attempts are being made to transform two of the cloned lines with chemical carcinogens. Treatment of the clones with N-methyl-N-nitro-N-nitrosoguanidine (1.0 and 0.1 µg/ml medium) has resulted in changes in morphology, an increased growth rate, and a prolongation of the life expectancy of the cells. The cultures are being monitored for the ability of the cells to grow in soft agar and to produce tumors in athymic, nude mice. Studies with radiolabeled benzo[a]pyrene (BP) showed that the bovine cells possess moderate amounts of aryl hydrocarbon hydroxylase activity, and metabolize benzo[a]pyrene (BP) to the 9-hydroxy, trans-7,8-diol, trans-4,5-diol, trans-9,10-diol, quiones, (7/8,9)-triol and two tetrol forms of benzo[a]pyrene. However, treatment of the cells with carcinogens that require metabolic activation: i.e., 7,12-dimethylbenzanthracene (@ 5.0, 1.0, 0.1, and 0.01 µg/ml) and diisopropylnitrosamine (@ 100, 50 and 10 µg/ml) either continuously or intermittently for periods of 3-4 months did not result in malignant transformation.

Significance to Biomedical Research and the Program of the Institute: Carcinoma of the pancreas is the fourth leading cause of cancer death in the United States, and its incidence is increasing. Although the causes of pancreatic cancer are unknown, recent epidemiologic studies have associated the disease with at least two variables; (a) excessive cigarette smoking, and (b) a diet high in fat and/or cholesterol. In addition, chemists and industrial workers exposed to such compounds as methylnitrosourethane, methylcholanthrene, and acetylaminofluorene have a higher incidence of pancreatic cancer than the general population. Given these results, it is important to develop experimental models for the study of chemical carcinogenesis in the human pancreas. Long-term maintenance of human pancreatic duct in explant culture has been achieved and attempts are being made to isolate and culture pancreatic epithelial cells. Bovine pancreatic duct tissues are being used as an in vitro animal model system for studies of chemical carcinogenesis. It is anticipated that methodologies developed in the bovine system might be applicable to the human tissues. These models should be useful for the study of chemical carcinogenesis in the pancreatic duct, and for the identification of host factors determining susceptibility to chemically induced pancreatic cancer.

Proposed Course of Project: Explants of human and bovine pancreatic duct have been cultured for extended periods in vitro. Biochemical studies have shown that these tissues have the ability to metabolize chemical carcinogens and experiments are underway to determine whether the epithelial cells can be transformed to malignancy with chemical carcinogens. Cells with an epithelial morphology have been isolated from bovine pancreatic duct and cultured in vitro for prolonged periods. The cells were cloned and the clones are being treated with carcinogens to determine whether they can be transformed to malignancy. Studies will be conducted to determine whether the growth of human pancreatic duct cells might be improved by examining the nutritional requirements of the cells; e.g., media, serum, etc., and by culturing the cells on different substrates; e.g., collagen, x-irradiated feeder layer, or gelatin sponge. After establishment of optimum growth conditions for the cells, carcinogenesis studies will be initiated.

Publications

Jones, R., Barrett, L., van Haaften, C., Harris, C. and Trump, B.: Studies on carcinogenesis in the pancreas. I. Long-term organ culture of bovine and human pancreatic ducts. J. Natl. Cancer Inst. 58: 557-565, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04487-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism of Chemical Carcinogens in Cultured Human Pulmonary Alveolar Macrophages

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Autrup	Staff Fellow	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
	G. Stoner	Research Scientist	EXP, NCI
OTHER:	J. Selkirk	Research Scientist	ORNAL, TN

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolism of benzo[a]pyrene (BP) by human bronchus and pulmonary alveolar macrophages (PAM) from the same donor was studied to investigate whether the PAM might be useful as an indicator cell for the human bronchus. PAM were able to enzymatically to convert BP into metabolites which bound to cellular macromolecules. Negative correlation was observed between aryl hydrocarbon hydroxylase and binding of BP to cellular protein. A 9-fold interindividual variation was found in the binding to DNA. The majority of the metabolites of BP were water-soluble, while 7,8-dihydro-7,8-dihydroxy BP (7,8-diol), 9,10-dihydro-9,10-dihydroxy BP, triols and tetrols were the major metabolites in the ethyl extractable fraction. PAM metabolize BP into its proximate carcinogenic form, 7,8-diol, which is released into the extracellular space. No correlation of AHH activity and binding to DNA or protein between PAM and bronchus was found.

PHS-6040
(Rev. 10-76)

Objective: To characterize the pathways of BP metabolism by cultured human PAM. To compare metabolism of BP in PAM with that of cultured human bronchus. To investigate the correlation if any between metabolism of BP by alveolar macrophages and bronchus isolated from the same donor.

Methods Employed: Explant culture of human bronchus and cell culture of PAM; enzyme assays; isolation of cellular macromolecules; high pressure liquid chromatography.

Major Findings: This is a new project. The metabolism of BP by cultured human bronchial epithelium and cultured PAM from the same donor was studied. Explants of human bronchus were prepared from surgical and autopsy specimens and human PAM were isolated from peripheral lung by trypsinization and by differential adhesion to plastic tissue culture dishes. After 7 days in culture the bronchus explant and the PAM were exposed to BP, and the binding to cellular macromolecules was studied. Aryl hydrocarbon hydroxylase activity was determined by the release of tritiated water into the culture medium from metabolized [^{3}H]-benzo[a]pyrene. A negative correlation between binding to protein and aryl hydrocarbon hydroxylase exists in pulmonary macrophages, but no correlation between data from bronchus and from macrophages was found. Variation in the binding level of BP to DNA and to protein in PAM from different individuals showed 9- and 33-fold interindividual variation, respectively. The metabolism of BP was further investigated in the macrophages. Both binding of BP to macromolecules and aryl hydrocarbon hydroxylase activity were dependent on the length of time the explant was in culture and on length of exposure to BP. Pretreatment of the macrophages with benz[a]anthracene increased both binding levels of BP and aryl hydrocarbon hydroxylase activty. When coincubated with BP, cycloheximide, 7,8-benzoflavone or actinomycin D reduced both the level of binding and the activity of aryl hydrocarbon hydroxylase. When PAM cultures were maintained at pO_2 greater than atmospheric air, an increase in binding level and aryl hydrocarbon hydroxylase activity was found.

The major ethylacetate extractable metabolites of BP formed by macrophages were 7,8-diol, 7,10/8,9-tetrol, and a peak containing 9,10-diol, 7/8,9,10-tetrol and 7/8,9-triol. An increase in the level of 7,8-diol was observed when PAM and human bronchus was cocultivated. About 50% of the metabolites were water-soluble, some of which were conjugated with sulphate and glucuronic acid.

Significance to Biomedical Research and the Program of the Institute: These preliminary results indicate that PAM might not be used as an indicator cell for human bronchus, the major site of lung cancer, and hence not be useful in determining an individual's risk to bronchogenic carcinoma by measuring enzyme levels in PAM. Since PAM metabolize BP to the proximate carcinogen, 7,8-diol, and release it into the extracellular space, PAM may play a role in preactivation of BP in the lung.

Proposed Course of the Project: Collect additional data on enzyme activity in PAM and bronchus from same donor to verify our preliminary results. Metabolism of other carcinogens by PAM will also be studied. PAM will be used as activating source for mutagenesis assays.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04488 01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism of Chemical Carcinogens in Cultured Human and Rat Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Autrup	Staff Fellow	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
OTHER:	J. Selkirk	Research Scientist	ORNAL, TN
	B. Trump	Chairman of Pathology	Univ. MD

COOPERATING UNITS (if any)

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LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	0.75	0.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Explant cultures of both human and rat colon have the ability to enzymatically convert various classes of chemical carcinogens, such as polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins and hydrazines, into metabolites which react with cellular macromolecules. The effect of various co- and anti-carcinogens of colon carcinogenesis on the metabolism of benzo[a]pyrene (BP) and of 1,2-dimethylhydrazine (DMH) by the rat colon has been studied. Different segments of the rat colon metabolize BP and DMH to varying extents. The metabolism of BP, less in the colon was approximately 10% of the level found in the human bronchus; the major metabolites, extractable with ethylacetate, were quinones, a peak containing 9,10-diol and 7/8,9-triol and 7,8-diol. The metabolite pattern of BP in human and rat colon were quite similar. Secondary bile acids significantly decreased the binding of DMH to rat colon, while enhancing the binding level of BP to DNA in human colon. Benz[a]anthracene did not enhance either activity of aryl hydrocarbon hydroxylase or BP binding to DNA in human and rat colon.

Objectives: To compare the metabolism of chemical carcinogens in human colon to that in experimental animals. To study the effect of various co- and anti-carcinogens on the metabolism of these carcinogens.

Methods Employed: Explant cultures of human and rat colon; enzyme assays; isolation of cellular macromolecules; high pressure liquid chromatography; autoradiography.

Major Findings: Cultured rat colon was able to metabolize benzo[a]pyrene (BP), 7,12-dimethylbenz[a]anthracene (DMBA), aflatoxin B_1 (AFB_1), dimethylnitrosamine (DMN), 1,2-dimethylhydrazine (DMH) and methylazoxymethanol acetate. 1,2-Dimethylhydrazine, an organotrophic carcinogen for the colon in rodents, had the highest binding level to DNA of the compounds tested. DMH methylated both the O-6 and N-7 position of guanine in DNA. Activity of aryl hydrocarbon hydroxylase (AHH) and binding levels of BP to macromolecules were higher in the descending colon when compared to the other segments of the colon. The major metabolites of BP, extractable with ethylacetate, were quinones, tetrols, 7,8-diol and a peak containing 9,10-dihydroxy-9-10-dihydrobenzo[a]pyrene and 7,8,9-trihydroxy-7,8-dihydrobenzo[a]pyrene. The binding levels of BP to DNA protein in the explant was lowered by co-incubation with 7,8-benzoflavone a known inhibitor of AHH, and with disulfiram, an antioxidant. The absence of vitamin A in the media also resulted in lower level of BP binding to DNA and to protein and in lower activity of AHH. Pretreatment with known inducers of AHH in other systems such as phenobarbital (PB) or benz[a]anthracene, did not have any significant effect on the binding levels of BP to DNA. Of the bile acids investigated only taurodeoxycholic acid significantly increased the binding level of BP to DNA.

The metabolism of DMH by rat colon was significantly decreased by co-incubation with bile acids. Pretreatment of the explants with PB significantly increased binding to both DNA and protein. Different segments of the colon metabolized DMH to different extents, descending transverse ascending rectum.

Cultured human colon could metabolize BP, DMBA, DMH, DMN, diethylnitrosamine and N-nitrosopyrrolidine. The metabolism of the polynuclear aromatic hydrocarbons was studied by determining binding to DNA and to protein and by activity of AHH and for BP the metabolite pattern which was measured by high pressure liquid chromatography. The metabolism of cyclic and acyclic nitrosamines was studied by both the release of ^{14}C-labelled CO_2 and binding to cellular macromolecules. Both DMN and DMH methylated N-7 and O-6 positions of guanine in DNA.

The metabolism of BP, DMH and AFB_1 by various segments of the colon from the same patient was investigated. The inter-individual variation in the binding to DNA of BP and DMH was 60-fold and 30-fold, respectively. 7,8-Benzoflavone and disulfiram, both inhibitors of the mixed function oxidases, inhibited the activity of AHH and the binding of BP to DNA and to protein, while secondary bile acids enhanced the binding to DNA and to protein.

Significance to Biomedical Research and the Program of the Institute: Chemical carcinogens which are metabolically activated and form carcinogen-DNA adducts by human colon may induce cancer in this tissue in humans. Determination of binding levels of carcinogens in a greater group could identify people at high risk for chemical induction of colonic cancer.

Proposed Course of the Project: Study in more detail the metabolism of BP and N-nitrosopyrrolidine e.g., identification of the DNA carcinogen adducts. The effect of various co- and anti-carcinogens on the metabolism of DMH will be investigated in human colon. The relationship between the genetic control of DMH-carcinogenesis and the enzymatic activation will be investigated in animal models.

Publications

Autrup, H., Harris, C. C., Stoner, G. D., Jesudason, M. L., and Trump, B. F.: Binding of chemical carcinogens to macromolecules in cultured human colon. J. Natl. Cancer Inst., In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04489-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Explant Culture of Human and Rat Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Autrup	Staff Fellow	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
	G. Stoner	Research Scientist	EXP, NCI
	L. Barrett	Instructor	Univ. MD

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Conditions for culturing explants of both human and rat colon has been developed. Rat colon has been maintained up to 35 days under these conditions, while human colon could generally be maintained up to 4 days; tissue from some patients could be maintained up to 10 days. The viability of the tissue was determined by incorporation of radioactive precursors into DNA and protein, and by electron microscopy.

PHS-6040
(Rev. 10-76)

Objectives: To develop an explant culture system of human colon to enable carcinogenesis studies in this organ under controlled conditions in vitro.

Methods Employed: Explant cultures; electron microscopy; high resolution light microscopy; autoradiography.

Major Findings: This is a new project. Explant culture systems were developed for both human and rat colon. For human colon, surgical specimens of non-tumorous human colonic tissue were obtained from patients with adenocarcinoma of the colon. Grossly normal appearing pieces were immediately immersed in L-15 medium and kept at 4°C until cultured. The specimens were then cut into approximately 0.75 by 0.75 cm squares and cultured in 60 mm plastic Petri dishes containing 2.5 ml of CMRL 1066 tissue culture media supplemented with L-methionine (1 µg/ml), hydrocortisone (0.5 µg/ml), β-retinyl acetate (0.1 µg/ml), penicillin G (100 U/ml), streptomycin (100 µg/ml), gentamicin (50 µg/ml), amphotericin B (0.25 µg/ml) tricine buffer (25 mM), L-glutamine (3 mM) and 5% bovine serum albumin Fraction V (Miles Laboratories, Elkhart, Ind.). Cultures were maintained in an air-tight chamber and gassed with 95%O_2 and 5%CO_2 for 5 min. The chamber containing the cultures was rocked at approximately 10 cycles/min so that the colonic tissue was submerged half of the time. Incubation temperature was 36.5°C. Human colonic tissue has been maintained up to 10 days under those conditions.

Similar culture conditions were used for rat colon which has been maintained for up to 35 days. One of the difficulties in culturing colon explants was excessive production of lactic acid. A major improvement in the system was accomplished by increasing the O_2-tension from 45% to 95%, which resulted in a decrease in latic acid production. Methionine was added to the media, as it is a constitutive part of mucous produced by the colon, and because amino acid analysis of the media showed depletion of this component.

Both electron and light microscopy and autoradiography were used for monitoring the cell viability, as well as incorporation of ^{3}H-Tdr and ^{3}H-Leu. Incorporation of ^{3}H-Tdr only takes part in the lower parts of the crypt, while ^{3}H-Leu is incorporated in all cells.

Significance to Biomedical Research and the Program of the Institute: A colon explant system has been developed which can be used for carcinogenesis studies and for studies of the pathogenesis of other colonic diseases.

Proposed Course of the Project: Improving the culture condition to be able to maintain the explant for a longer time. Attempts will be made to culture dispersed epithelial cells from rat and human colon.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04490-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Evaluation and Prevention of Carcinogenic Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Umberto Saffiotti, M.D. Chief, Experimental Pathology Branch
CG, DCCP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary objectives of this work are to examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic effects of chemical and physical agents and for the prevention of cancer hazards in the human population.

Laboratory methods and biological models for the detection of carcinogenic activity of chemicals are examined and evaluated, with particular emphasis on in vitro models for carcinogenesis studies and animal models of carcinogenesis.

Criteria for the evaluation of carcinogen risk from environmental and occupation human exposures are developed.

PHS-6040
(Rev. 10-76)

Objectives: To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic effects of chemical and physical agents and for the prevention of cancer hazards in the human population.

Methods Employed: Examination and evaluation of biological models, methods and findings used for the detection of carcinogenic activity. Analysis of results of carcinogenesis studies, including pathology, experimental design and statistical evaluations. Analysis of occupational and environmental exposure data. Analysis of regulatory and legislative positions. Development of critical reviews and documentation in environmental carcinogenesis. Organization of conferences and workshops to provide evaluation of current scientific bases of carcinogenesis studies. Preparation and editing of documentation on these topics. Participation to advisory groups to national and international organizations and to agencies of Federal Government.

Major Findings: Particular contributions were provided in the following areas:

1. Documentation on in vitro carcinogenesis methods: (a) Organization of a "Conference and Workshop on In Vitro Carcinogenesis" (Aspen, July 1976) which reviewed major advances in this field. (b) Editing and publication of a book entitled "In Vitro Carcinogenesis. Guide to the literature, recent advances and laboratory procedures" (U. Saffiotti and H. Autrup, Eds.; NCI Carcinogenesis Techn. Rep. Series, 1977, in press). (c) Organization of a Workshop on Human Tissue Studies in Carcinogenesis, with C. C. Harris and B. F. Trump (Aspen, August 1977), the first laboratory workshop devoted to a critical evaluation of the new methods for carcinogenesis studies directly on human tissues.

2. Definition of criteria for carcinogen identification and risk assessment. In a series of papers presented at scientific conferences and prepared for publication, the following general criteria were defined: (a) distinction of toxic effects into terminal toxic effects and self-replicating toxic effects; (b) classification and definition of carcinogenesis test results into the categories of "positive", "negative under the conditions of observation" and "inconclusive"; (c) distinction of the process of risk evaluation from the process of benefits evaluation and need for a third process of evaluation of technological alternatives for carcinogenic hazard prevention; (d) ethical implications of knowledge on etiology; (e) need for toxicology standards comparable to those required in forensic medicine; (f) the need for licensed laboratories for carcinogenesis testing; (g) the need for full public documentation of carcinogenesis data.

3. Review of state of knowledge and recommendation of criteria for the evaluation of the carcinogenic effects of air pollution. Working papers were prepared for a conference convened by the Karolinska Institute in Stockholm, March 8-11, 1977; a final report was prepared for publication, with recommendations to the Swedish Government.

4. Advisory activity to Agencies of the federal government on the evaluation of regulatory criteria for environmental carcinogens. (a) Participation at the request of the Assistant Secretary of Labor for OSHA in the drafting of the proposed "Regulation of certain toxic materials. Identification, classification and regulation of toxic materials posing a potential occupational cancer risk to workers". This proposed regulation provides, for the first time in the USA, for a comprehensive occupational control of carcinogenic hazards, and is based on different degrees of scientific evidence of carcinogenicity. (b) Evaluation and submission of testimony for administrative law hearing, at the request of the Office of the General Council, Food and Drug Administration, on the evaluation of the carcinogenicity of diethylstilbestrol. (c) Advisory activity to the Administrator, Environmental Protection Agency, in the search for candidates for the position of Assistant Administrator for Toxic Substances and of Chairman, Cancer Assessment Group. (d) Presentations to conferences on the Federal regulation of carcinogens, Toxic substances Law, Public Information on the Prevention of Occupational Cancer (NRC), Women in the Workplace.

(5) Historical review of the progress in Carcinogenesis studies at NCI, 1957-77, for publication in the 40th anniversary issue of JNCI.

(6) Invited participation to scientific and advisory panels: Conference on the Future of Academic Pathology (rapporteur on Cancer panel) UAREP, September 1976; American Cancer Society advisory group on environmental cancer, October 1976; Conference on dimethylcarbamoyl chloride, New York University, October 1976. Meeting of the section on Occupational Medicine, Royal Society of Medicine, London, March 1977; conference on mechanisms of Tumor Promotion and Cocarcinogenesis, Oak Ridge Natl. Laboratory, March 1977. Also participation to the scientific activities of the Society for Occupational and Environmental Health, as Vice-President.

Significance to Biomedical Research and the Program of the Institute: The national policies on environmental health and cancer prevention should be based on sound scientific grounds. A solid and well documented basis on research experience and evaluation is of great value in the development of criteria for a sound health protection policy.

The coordination of different methodological approaches to the study of the carcinogenic process, at the human, animal, cellular and molecular level, provides a stronger basis for focusing on specific research directions with high priority.

Proposed Course of Project: Continuation of these activities.

Additional Information: This new project is in partial continuation of previous Project No. Z01-CP-04696-06-CG.

Publications

Saffiotti, U.: Validation of short-term bioassays as predictive screens for chemical carcinogens. In Montesano, R., Bartsch, H., and Tomatis, L. (Eds.) Screening Tests in Chemical Carcinogenesis. Lyon, France, International Agency for Research on Cancer, IARC Scientific Publ. No. 12, 1976, pp. 3-13.

Saffiotti, U. and Wagoner, J. K. (Eds.): Occupational Carcinogenesis. Ann. N.Y. Acad. Sci. 271: 516, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04491-01 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Quantitative studies on concurrent exposures to numerous carcinogens.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Umberto Saffiotti	Chief, Experimental Pathology Branch	EXP, NCI
OTHER:	Jerry Rice	Head, Perinatal Carcinogenesis Section	EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this new project it is planned to develop an experimental design for the concurrent exposure of appropriate biological targets to numerous carcinogens of different classes, each administered at doses which would be expected to produce marginal or undetectable effects in the chosen system. Quantitative exposure/response studies will be addressed to test the hypothesis that many different carcinogens can act synergistically to induce a significant level of carcinogenic response when given concurrently at individually ineffective doses. The methods to be used include in vitro assays for mutagenesis and neoplastic cell transformation and whole animal models.

PHS-6040
(Rev. 10-76)

Objectives: To determine whether a marked carcinogenic effect can result from the concurrent exposure to numerous different carcinogens, each administered at doses which would be expected to produce marginal or undetectable effects in the chosen target system.

Methods Employed: Biological systems to be used include Salmonella mutation tests (Ames), neoplastic transformation of cells in culture and eventually whole animal carcinogenesis. Quantitative exposure protocols will be developed. It is planned to extend the exposures to as many as 100 different carcinogens given concurrently. Quantitative analysis of the results will be performed.

Significance to Biomedical Research: Multiple concurrent exposures to many different carcinogens represent the actual conditions under which the human population is exposed to carcinogens. From prenatal life through childhood and adult life people are exposed to a large number, probably in the hundreds, of environmental carcinogens from different routes. Such "realistic" conditions of exposure have never been reproduced experimentally so far. The definition of the effect of this background low-level exposure to individual carcinogens, acting together with the exposure to many others, is expected to provide information on the mechanisms of carcinogenesis in tissues exposed to multiple hits by different chemicals and on the role of multiple synergisms in carcinogenesis. The role of high-level exposures to single carcinogens when superimposed to different kinds of background multiple exposures will also be clarified. Wide-ranging implications can be projected for the understanding of basic chemical biological interactions in carcinogenesis, for mathematical models for dose/response extrapolation and for the evaluation of carcinogenic hazards in public health policies.

Proposed Course of Project: Continuation.

Publications

An outline and discussion of this project is included in the following publication:

Saffiotti, U.: Identifying and defining chemical carcinogens. In Hiatt, H., Watson, J. D., and Winsten, J. (Eds.): Origins of Human Cancer. Cold Spring Harbor Conferences on Cell Proliferation. Cold Spring Harbor Laboratory, N.Y., 1977, Vol. 4, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04492-01 EXP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of irradiation on tumors in Mastomys natalensis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Goodman	Veterinary Pathologist	EXP NCI
	J. D. Strandberg	Veterinary Pathologist	

COOPERATING UNITS (if any)

Johns Hopkins University, School of Medicine

LAB/BRANCH

Experimental Pathology Branch, Carcinogenesis Program

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mastomys natalensis develop a high incidence of spontaneous thymomas and gastric carcinoids in addition to a variety of other neoplasms. The animals were irradiated at an early age to study the effect of irradiation on the development of tumors in this species. Currently, the treated animals are showing clinical signs of early aging. However, it is too early to evaluate the effect of treatment on the development of tumors.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine the effect of x-irradiation on the development of tumors in Mastomys.

Methods Employed: Male and female Mastomys natalensis were irradiated with 900 rads split into 2 doses given at 6 week intervals. The animals are being observed daily for clinical signs and the appearance of tumors. Animals are killed when found moribund. All animals found dead or killed will be autopsied and examined histopathologically.

Major Findings: The irradiated animals are somewhat smaller than the non-treated animals and are exhibiting alterations in hair coat color. However, it is too early to evaluate the effect of treatment on the development of tumors.

Significance to Biomedical Research and the Program of the Institute: Mastomys develop some unusual tumors, namely thymomas and gastric carcinoids for which there are no other good animal models. It is of interest if the age of onset or incidence of these tumors can be altered by irradiation.

Proposed Course of Project: Continuation.

Publications

1. Priester, W.A., Goodman, D.G., Theilen, G.H.: Nine simultaneous primary tumors in a boxer dog. J. Am. Vet. Med. Assoc. 170:823-826, 1977.

2. Squire, R.A., Goodman, D.G., Dawe, C.J., Frederickson, T., Harshbarger, J. C., Levitt, M.H., Lingeman, C.H., Strandberg, J.D., and Valeri, M.G.: Tumors. In Garner, T.C., Benirschke, K., Jones, T.C., and Kozma, C. (Eds.): Pathology of Laboratory Animals. New York, Springer-Verlag (In Press).

3. Morton R.W., Goodman, D.G., Gershwin, M.E., Squire, R.A., Steinberg, A.D.: Suppression of Autoimmunity and Lymphoid Proliferation in NZB mice with Steroid-Sensitive X-radiation-Sensitive Syngeneic Young Thymocytes. Science (In Press).

4. Goodman, D.G., Altman, N.H.: Table on Spontaneous Tumor in Inbred Rats. In Altman, P.L., Katz, D.D. (Eds.): Inbred and Genetically Defined Strains of Laboratory Animals Handbook. FASEB (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04503-05 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Anti-tumor immunity in transplacental induction of mouse lung tumors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rice	Senior Scientist	EXP, NCI
OTHER:	W. J. Martin	Research Med. Officer	Bureau Biologics, FDA
	T. G. Gipson	Research Associate	Bureau Biologics, FDA

COOPERATING UNITS (if any)

Division of Virology, Bureau of Biologics, FDA

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Perinatal Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transplantation immunity to transplacentally induced mouse lung tumors is related to a cross-reactive antigen present on many lung tumors of strains C3Hf, C57BL/6 and DBA/2 mice. Genetic control of antigen expression is closely linked to the H-2 histocompatibility locus. The role, if any, of this antigen in genetic control of susceptibility and resistance to lung tumors in mice, and its potential exploitability for immunotherapy and immunoprophylaxis of such tumors, is under study.

PHS-6040
(Rev. 10-76)

Objectives: To determine the manner and the extent to which immunological factors are responsible for the genetically determined differences in sensitivity to the induction and growth of epithelial tumors among different inbred strains of mice, and between the fetus and adult within each strain.

Methods Employed: Tumors are induced transplacentally by treatment of timed-pregnant mice with 1-ethyl-1-nitrosourea (ENU), which is synthesized in this laboratory. Tumors are transplanted to secondary hosts using trocar implants or counted cell suspensions, and to serial cell culture using standard media and procedures. Microcytotoxicity tests are carried out using lymph node cells from tumor bearing and tumor immunized mice. In vivo studies of immunological cross-reaction are carried out by pre-immunizing mice with normal or tumor tissue, subsequently suppressing the primary (but not the secondary) immune response by 400 R whole-body X-irradiation, and challenging with the tumor to be tested.

Major Findings: It has been previously reported that certain pulmonary tumors, induced transplacentally by ENU in strain C3Hf/HeN mice, grow poorly or not at all on transplantation to syngeneic recipients, but grow readily in F_1 hybrids between C3Hf/HeN and A/HeN mice. These tumors have subsequently been shown to be cross reactive, by the criterion of inhibition of in vivo growth of challenge inocula, with each other, with normal tissues from most organs of strain A and certain other strains of mice, and with some but not all transplacentally induced lung tumors from strains DBA/2N, C57BL/6N, and C3Hf/HeN. The tumors do not cross-react with normal tissues from fetal or adult mice of these 3 strains.

By the use of F_1, F_2, and backcross progeny of mice bred from strain A and C3Hf, it has been shown that progressive tumor growth following transplantation occurs only in progeny which have inherited the H-2^a haplotype, and from the proportions of F_2 and backcross animals in which tumor transplants succeeded it is inferred that permissive tumor growth is due to a single autosomal genetic locus which is closely linked to the locus controlling the expression of the H-2^a haplotype in strain A mice. Immunization of C3Hf mice, prior to irradiation, with normal tissues from congenic BC57BL/10J (B10;H-2^b) and B10.A (H-2^a) mice conferred antitumor resistance only in recipients of B10.A tissue, indicating that a product of a gene closely linked to the H-2 locus is cross-reactive with and possibly identical to the tumor antigen. Further studies with normal tissues of the B10.A (2R) recombinant, which expresses only the K region of the H-2^a haplotype, and the B10.A (5R) recombinant, which express the D end of that locus, showed that the gene that codes for the tumor antigen is further localized to the K end of the H-2 histocompatibility locus.

Significance to Biomedical Research and the Program of the Institute: The existence of cross-reactive antigens which confer transplantation resistance on tumors and normal lung tissue of certain individuals invites the question of whether a similar mechanism may explain tolerance (susceptibility) to other types of tumors in animals or in human beings. This in turn has significance for efforts to develop immunotherapeutic measures against neoplastic disease.

This experimental model provides a means for determining whether non-specific stimulation of immune responsiveness will suffice to initiate tumor rejection in individuals who are intrinsically tolerant to tumor antigens.

Proposed Course of Project: Recent data indicate that the transplantation antigen is not present on fetal tissue of C57BL/6 or DBA/2 mice. It is thus possible that the antigen is not expressed routinely during ontogeny and is derepressed in the process of malignant transformation. Experiments will determine if the antigen is expressed in other situations; these include the period after ENU administration but before malignancy is histologically detectable. The relation of the tumor antigen to normal gene products is being explored and cross-reactivity studies are being extended to determine whether the antigen is present on normal and/or tumor tissue from rats, guinea pigs, rabbits, or humans. The cross-reactivity demonstrated on the 85 lung tumor model will be exploited in both immunoprophylactic and immunotherapeutic protocols.

Publications

Martin, W. John, Gipson, Thomas G., and Rice, Jerry M.: H-2^a-associated alloantigen expressed by several transplacentally-induced lung tumours of C3Hf mice. Nature 265: 738-739, 1977.

Martin, W. J., Gipson, T. G., Martin, S. E., and Rice, J. M.: Depressed alloantigen on transplacentally induced lung tumor coded for by H-2 linked gene. Science 194: 532-533, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04504-05 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Model Systems for the Study of Chemical Carcinogenesis at the Cellular Level.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Yuspa	Head, In Vitro Pathogenesis Section	EXP, NCI
OTHER:	H. Hennings	Senior Scientist	EXP, NCI
	U. Lichti	Expert	EXP, NCI
	N. Colburn	Expert	EXP, NCI
	T. Bowden	Staff Associate	EXP, NCI

COOPERATING UNITS (if any)

Differentiation Control Section, Experimental Pathology Branch

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
In Vitro Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.5	5	4.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mouse epidermal cell cultures have been utilized as a model for studying mechanisms of epithelial carcinogenesis in vitro. Untreated cells have a limited lifespan in culture while carcinogen treated cells form longterm lines, some of which are tumorigenic in animals. Carcinogens induce both excision repair and post-replication repair in these cultures and by use of a radioimmunoassay the fate of the carcinogen-nucleoside adduct formed in culture can be followed. Promoters stimulate ornithine decarboxylase activity and DNA synthesis after brief exposure. Exogenous polyamines enhance the proliferative response while steroids inhibit it. The steroid appears to act by decreasing the cells ability to respond to polyamines and its effects can be overcome by exogenous polyamines.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To develop model systems which can be utilized to study cellular events which occur during stages of chemical carcinogensis. Systems studied are directed to give both general information regarding malignant transformation within a mammalian cell and specific requirements of specialized cells which might shed light on events necessary for tumor development in a particular organ. In addition specific markers of the transformed phenotype are being sought and mechanisms to prevent or reverse transformation are being studied.

Methods Employed: Cell culture systems have been developed for studying mechanisms of chemical carcinogenesis in epithelial cells. The system developed in our laboratory which is most extensively studied has been mouse epidermal cell culture. This system closely mimics the classical mouse skin carcinogenesis model in vivo and where necessary appropriate parallel in vivo experiments have been done to validate in vitro findings. During the past year studies have also been undertaken in human epidermal cells taken from foreskins. Such cultures have a striking similarity to mouse epidermis in vitro and appear to have a longer lifespan in the differentiated state in culture. Using these culture systems a number of parameters are studied following treatment with carcinogens, promoters or mediators of carcinogenesis. Evaluation of results utilize the techniques of light and electron microscopy and more recently fluorescence microscopy.

Alterations in growth kinetics are followed by biochemical and autoradiographic techniques. Altered cell functions are studied by enzymatic assays, precursor uptake and electrophoresis. The assumption of the malignant phenotype is monitored by soft agar assays, growth rates, production of plasminogen activator and injection of cells into mice. Several immunologic techniques including cell surface antibody production, fluorescent staining, cytotoxicity assays and mixed hemabsorption are being utilized to recognize altered phenotype. Additionally antibody techniques including radioimmunoassay have been developed to monitor interaction of carcinogens with DNA. DNA repair is being studied by density gradient centrifugation and molecular weight determinations following carcinogen damage.

Major Findings: Continued efforts to characterize and improve the culture conditions for mouse epidermal cells and tranformation have revealed that various commercial media make little difference in morphology or growth kinetics. Serum concentration of 10% appear optimum at 37^{o} but more rapid proliferation occurs at 2% serum followed by enhanced keratinization. Lower temperature (31^{o}) prolongs culture life while slowing growth rate. Epidermal growth can be essentially stopped at pH 6.7 and accelerated at pH 7.6. At the lower pH differentiation also appears to be inhibited. Growth of Balb/c epidermal cells on irradiated or ethanol fixed fibroblast monolayers enhances plating efficiency by about 50% and prolongs the ability to subculture by about 2 fold over plastic substrate (4-5 passages vs 2 passages). Epidermal cells obtained from SENCAR mice (bred for susceptibility to skin carcinogenesis and yielding approximately 30 more tumors/mouse than Balb/c) grow on

all substrates equally well and can be subcultured for more than 5 passages in all cases but are not tumorigenic. Balb/c cell under all growth conditions occasionally undergo spontaneous conversion to long term growth (4 lines/34 primary cultures) but such lines are not tumorigenic. MNNG treated Balb/c cells are yielding long term lines in most cases and some of these are tumorigenic and grow in agar. Quantitation of these findings and dose response relationships are now in progress. Several of these long term non-tumorigenic lines, both MNNG treated and untreated, are being exposed to promoting agents to ascertain if they are stable initiated cells. In one case conversion from agar negative to agar positive has occurred (1 of 4 lines tested) within 3 weeks of promoter treatment. In order to develop markers for normal and transformed phenotypes, antibodies have been made against epidermal cell surfaces of normal and transformed cells and the keratin proteins produced in vitro by epidermal cells. Cell surface antibodies are produced in high titer as determined by mixed hemadsorption testing. Human epidermal cells grow exceptionally well on a plastic substrate and can be subcultured at least 2 times. Studies on growth kinetics and response to carcinogens are just beginning.

The interaction of carcinogens with the mammalian genome have been studied in two ways. Excision repair and post-replication repair after ultraviolet light and MNNG were determined by density gradient techniques. SENCAR epidermal cells appear to have a reduced repair capacity of UV light damage compared to Balb/c. The tumor promoter TPA inhibited repair when given 24 hours before UV damage but had no effect when given 96 hours before or immediately after. Also it was found that TPA did not alter DNA elongation rates at 24 or 96 hours after exposure, times when DNA synthesis is either markedly inhibited or stimulated respectively. This implies that altered replication rates must be due to differences in initiation of replication. Additionally repair studies have demonstrated that MNNG induces gaps in replicating DNA which can be rapidly filled, but this process can be inhibited by caffeine. Over the past year a second approach to probing carcinogen-DNA interactions has been developed. Antibody has been developed against the purine adduct of the carcinogen N-acetoxy-acetylaminofluorene (NA-AAF) which binds to the C-8 position of deoxyguanosine (G-AAF). Using this antibody a radioimmunoassay was developed, sensitive enough to detect 0.5 - 1 nanograms of G-AAF. This antibody has been purified by affinity chromatography. The assay has been able to detect G-AAF in 50 ug of DNA reacted with NA-AAF in vivo and in chemically modified M. Luteus DNA and appears to be quite specific for the adduct.

A major effort has been directed toward the study of tumor promotion since this is a potentially reversible phenomenon. Mouse epidermal cell cultures appear to be an excellent model for assay and mechanistic studies of tumor promotion. Brief exposures to small doses of phorbol esters stimulate ornithine decarboxylase (ODC) activity 10-15 fold and DNA synthesis 5-15 fold. Both of these effects occur in vivo also. Exogenous putrescine enhances the proliferative response in a dose dependent fashion. Several classes of promoting substances can be detected by the ODC assay including phorbol esters, anthralin, polychlorinated biphenyls and tweens. Interestingly SENCAR mice show only a 3-4 fold induction of ODC after TPA but both basal ODC levels and

proliferation rate were higher than Balb/c. S-adenosyl methionine decarboxylase activity has also been assayed after TPA. Unlike the in vivo situation, the activity of this enzyme is depressed for up to 5 days after treatment suggesting again that putrescine is the polyamine involved in the proliferative response to TPA. Manipulation of culture conditions have revealed that the TPA response for stimulated DNA synthesis requires serum to be present after TPA treatment but not before. Serum levels of 10% are optimum. At pH 6.7 the ODC response is depressed and the proliferative response is eliminated. A surprising result was obtained when cells were exposed to the antipromoting steroid fluocinolone acetonide (FA) prior to TPA. This drug, which completely inhibits tumor promotion and promoter stimulated proliferation in vivo, also inhibits stimulated proliferation in vitro. Paradoxically it potentiated the ODC response to TPA in vivo and in vitro. In vitro, the inhibition of TPA induced proliferation could be reversed by adding exogenous putrescine to the medium. These results suggest that putrescine is the mediator of promoter induced proliferation and steroids act by reducing cellular sensitivity to putrescine.

When retinyl acetate is added to the culture medium epidermal cell differentiation shifts from a keratinizing to a secretory epithelium. This appears to be mediated through remarkable alterations in glycoprotein synthesis. The findings in these studies are reported in detail in the Project Description of the Differentiation Control Section (Z01-CP-04798-07-EXP).

Significance to Biomedical Research and the Program of the Institute: The great majority of human cancers are induced by chemicals and most of these tumors develop in epithelial cells. Animal models have been extremely useful for bioassay and mechanistic studies but suffer from complicated interactions between host and environment as well as variations in physiological balance which impede investigations at the cellular level. The use of cell culture systems, particularly those of epithelial origin, offers the opportunity to extend the present state of knowledge to a more basic and cellular level. In addition cell cultures ultimately should be useful as bioassay screening procedures to detect carcinogens and co-carcinogens in the environment. Most of our present basic concepts concerning the pathogenesis of cancer were developed from studies utilizing carcinogen painting on mouse skin. The irreversibility of initiation, the phenomenon of co-carcinogenesis and tumor promotion, the role of hyperplasia and metabolism are examples of such concepts. The development of a cell culture system for epidermal carcinogenesis has been a major advance toward extending our knowledge of mechanisms of carcinogenesis. Earlier efforts were directed to proving that epidermal cells in culture responded to carcinogens and promoting agents as in vivo. In almost every parameter studied this was the case. Differentiation, metabolism, proliferation, activation and binding of carcinogens, promoter responses were highly analogous to in vivo. These findings enhanced the validity of any subsequent observations made in vitro. In the past year the model system has been utilized to a much greater degree to ask questions about mechanisms of transformation, carcinogen and promoter interactions and the role of anti-carcinogenic agents such as retinoids and steroids. Long term growth of non-tumorigenic and tumorigenic cell strains offer the opportunity to study the

cancer phenotype and tumor progression. Antigenic markers should be useful to detect both normality and abnormality and provide a rapid assay for each state. The use of cells from sensitive and resistant animals offers the opportunity to determine the factors responsible for susceptibility. The induction of ODC activity can detect several different classes of promoting substances and appears useful as bioassay. In mechanistic studies the role of ODC and polyamines classifies the promoter response in a category with a number of pleiotropic responses already known and defines a future approach based on sound scientific knowledge. The delineation of the role of antipromoter steroids interfering with polyamine sensitivity has implications beyond the scope of carcinogenesis but even within the cancer field it opens possibilities for understanding the role of steroids in chemotherapy. DNA repair studies in a relevant system enhance our understanding of the role of damage and repair of the target cell genome in the hope of intervening in the process. The development of an immunoassay for carcinogen-DNA adducts should have a number of important uses such as detection of carcinogens in human populations, following the repair of carcinogen damaged DNA and localizing the damaged site by a combination of immunological and morphologic techniques. Finally while bridging the gap between relevant animal models and in vitro systems, this laboratory is simultaneously developing the analogous human tissue model in vitro. Thus ultimately a chain of systems will be available to determine the relevance of findings in any one model for the entire spectrum of models including the human.

Proposed Course of Project: The quantitation of the transformation assay is still the major problem to be solved in the mouse epidermal cell culture system. Although advances in this area have been made in the preceeding year an even more vigorous effort will be made in the coming year. Preliminary evidence for the use of a pretreatment protocol with promoters and polyamines indicates that clonal growth may be possible in primary cultures and this avenue will be pursued. As data is collected in the mouse system similar experiments will be started in the human system. Wherever possible in vitro data will be correlated with similar experiments in vivo. Additional studies will involve the growth of initiated mouse epidermal cells obtained from mice painted with carcinogens in vivo. Additional markers of transformation will be studied. Excision and post-replication repair studies will be continued in an attempt to understand these processes and ultimately their role in transformation will be investigated utilizing inhibitors or enhancers of repair. The radioimmunoassay will be utilized to follow the persistence or repair of G-AAF with time and to determine if there is specific or random localization of the carcinogen in the genome.

Studies on the mechanism involved in tumor promotion will focus more on the role of polyamines, utilizing inhibitors of polyamine metabolism as well as steroids. In addition other model systems of polaymine mediated growth stimulation will be utilized to determine if the anti-carcinogenic activity of steroids is mediated through their interference with polyamine effects.

The role of retinoids in differentiation and carcinogenesis is an important area of research which is well suited for the model systems utilized in

this laboratory. In collaboration with the Differentiation Control Section, this laboratory will continue its investigations into retinoid alterations of glycoprotein synthesis and cell membrane function. The role of retinoids in promoter responses will also be studied.

Publications

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Yuspa, S. H., Hennings, H., and Saffiotti, U.: Cutaneous chemical carcinogenesis: past, present and future. J. Invest. Dermatol. 67: 199-208, 1976.

Edwards, W. D., Bates, R. R., and Yuspa, S. H.: Maintenance of epithelium in ventral and dorsal prostate in organ culture. J. Invest. Urol. 14: 1-4, 1976.

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Yuspa, S. H., Ben, T., Patterson, E., Michael, D., Elgjo, K., and Hennings,,H.: Stimulated DNA synthesis in mouse epidermal cell cultures treated with 12-O-tetradecanoyl-phorbol-13-acetate. Cancer Res. 36: 4062-4068, 1976.

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Yuspa, S. H., Elgjo, K., Morse, M. A., and Wiebel, F. J.: Retinyl acetate modulation of cell growth kinetics and carcinogen-cellular interaction in mouse epidermal cell cultures. Chem. Biol. Interact. 16: 251-264, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04505-03 EXP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pathology of Human Environmental Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Carolyn H. Lingeman Medical Officer EXP NCI

COOPERATING UNITS (if any)

Scientific coordinator for Environmental Cancers, NCI, Registry of Experimental Cancers, NCI, Armed Forces Institute of Pathology, other governmental agencies and private institutions.

LAB/BRANCH

Experimental Pathology Branch, Carcinogenesis Program

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major emphasis during the year has been on carcinogenesis associated with hormonally active compounds, mainly those induced in man and animal by diethylstilbestrol and synthetic estrogens and progestions composing oral contraceptives.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: 1) To define the epidemiologic, behavioral and morphologic characteristics of human malignant neoplasms known or suspected of being caused by carcinogens in the environment.

2) To provide pathology support for scientists of the NCI and other agencies and institutions.

Methods Employed: Pathologic materials (histologic slides, paraffin blocks, fixed pathologic specimens and other materials) from humans and animals with cancers and related lesions associated with documented exposure to environmental carcinogens are being collected. These materials are being indexed and filed and are available for examination by pathologists who visit the Department or by means of loan sets. The close proximity to the Registry of Experimental Cancers makes it possible to compare human and experimental lesions induced by the same carcinogens. For example, a current comparative study involves human DES-associated carcinomas of vagina and cervix and those of mice from the experiments of Dr. Thelma Dunn from the collection of the Registry of Experimental Cancers. Also available through the Registry is a set of slides of rats exposed to DES in utero from the experiments of Dr. Katharine Snell. Other similar comparative studies include pathologic effects of other environmental carcinogen as vinyl chloride, thorotract, arsenic and asbestos.

These materials are being used to prepare scientific articles, monographs and atlases to define and illustrate the morphologic characteristics of cancers, precancers and related lesions associated with documented exposure to specific carcinogens in the environment.

An exhibit entitled "Environmental Cancers" has been built for display at scientific meetings. Small, compact, light weight, this table top exhibit is composed of 3 separate sections that can be used together or individually. The material to be displayed is mounted on removable strips that can be replaced and modified from time to time. The top interchangable panels are lighted from behind to display photographs (gross and microscopic) of pathologic lesions. The bottom interchangable panels are for diagrams, tables and other written information.

Subjects of individuals panels completed so far are:

1. Carcinogens in Food - photographs of pathologic lesions in animals induced by carcinogens such as aflatoxins, cycasin and other compounds that may be present in food, beverages or drinking water.

2. Carcinogenic Hormones - photographs of preneoplastic and neoplastic lesions of liver and genitalia of human patients and animals exposed to estrogens (DES and those in oral contraceptives) and anabolic steroids.

3. Occupational Cancers - pathologic lesions of man and animals exposed to vinyl chloride, asbestos, nickel compounds and arsenicals (human only.)

Other panels to be prepared include "Drugs as carcinogens" and "Metals as Carcinogens."

A series of workshops, symposia etc. will attempt to summarize information about pathologic lesions associated with specific carcinogens. These workshops will be held at the NCI or at regular meetings of pathology organizations as part of the programs on continuing education as those of the International Academy of Pathology and other organizations. The first workshop will be devoted to Pathologic Lesions of Human Patients and animals exposed in utero and during infancy to DES.

A monograph entitled "Carcinogenesis of Some Hormonally Active Compounds" will be published during 1977 by Springer Verlag as one of their series "Recent Results in Cancer Research." It will be composed of papers presented at 2 1976 meetings of the DCCP's Interagency Collaborative Group on Environmental Carcinogenesis. These include papers presented by pathologists, epidemiologists, toxicologists and other scientists of the Armed Forces Institute of Pathology, the National Cancer Institute, the Center for Disease Control, the Food and Drug Administration, and the Frederick Cancer Research Center and cover a wide range of topies including cancers and related lesions of the liver, genitalia, mammary glands and other organs of human patients and animals exposed to DES, oral contraceptives, anabolic steroids and other hormones.

Major Findings: 1) Human DES-associated clear cell carcinoma of vagina and cervix should have anticipated few results of experiments in animals dating back to 1939 when Gardner et al and in 1963 when Dunn et al reported invasive cancers of cervix and vagina of female mice and malformation of genitalia of male and female mice exposed to DES during infancy. Rats exposed to DES in utero did not develop similar lesions.

2) Hepatocellular neoplasms in young women using oral contraceptives likewise should have been anticipated if results of experiments in rats published in 1972 had been heeded. Some adenomas in these young women appear very similar pathologically to those observed in rats exposed to various chemical carcinogens.

3) Several government agencies and private institutions have ongoing projects involving various aspects of hormonal carcinogensis but there is a lack of effective communication between all these investigators, particularly where the NCI is concerned. A major effort has been made to attempt to identify the many ongoing studies and to assess the capabilities as far as the pathologic documentation is concerned. Preliminary evidence indicate a lack of adequate pathologic documentation in many studies of human and animal neoplasms known as suspected of being induced by hormones.

Significance to Biomedical Research and the Program of the Institute: Pathology confirmation is required for clinical, epidemiologic and experimental carcinogenesis studies. The human environment is particular complex and, except for some occupational situations, and drugs prescribed in hospitals, exposure to suspected carcinogens is difficult to document. Human mortality data based on death certificates is particularly subject to error. Furthermore neoplasms which are rare in man, such as angiosarcomas, are sometimes misdiagnosed because pathologists rarely accumulate adequate numbers to gain experience in recognizing their characteristics. Information and pathologic specimens collected for this study provide material for use in workshops for preparation of exhibits, scientific papers, monographs and atlases to illustrate and summarize the morphologic characteristics of established environmental cancers, precancers and related lesions. While much of this information is available, though scattered, in standard sources as journals, medical textbooks and Tumor Atlases published by the Armed Forces Institute of Pathology and the World Health Organization, there is no single source summarizing the pathology of cancers in man and animals related to specific carcinogens in the environment. These activities are essential to increase the awareness of carcinogens in the environment and to encourage practicing pathologists to search for additional evidence of associations of specific types of cancers with exposure of the patient to potentially carcinogenic agents.

Proposed Course of Project: Efforts to increase the awareness of environmental carcinogens and their relation to human cancer will continue as information and pathologic materials from individuals with cancers associated with specific agents are collected and made available to practicing pathologists and other physicians and scientists. Pathologists with access to materials from patients with cancers and related lesions associated with exposure to carcinogens in the workplace or other area of the environment are being encouraged to contribute histologic slides, paraffin blocks, photographs and clinical information for this study. Epidemiologists at the NCI, NIOSH, CDC and other agencies and institutions investigation cancers of environmental origin are also being encouraged to obtain pathologic materials for this study.

Publications

1. The Vinyl Chloride Story. Bulletin of the Society of Parmacological and Environmental Pathologists. 4:9-15, 1976.

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3. Squire, R.A., Goodman, D.G., Dawe, C.J., Frederickson, T., Harshbarger, J.C., Levitt, M.H., Lingeman, C.H., Strandberg, J.D., and Valerio, M.G.: Tumors. In Garner, T.C., Benirschke, K., Jones, T.C., and Kozma, C. (Eds.): Pathology of Laboratory Animals. New York, Springer-Verlag (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04512-03 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Morphogenesis of Pancreatic Adenocarcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Levitt	Research Pathologist	EXP, NCI
OTHER:	C. C. Harris	Research Scientist	EXP, NCI
	R. A. Squire	Expert Consultant	EXP, NCI*
	E. Kingsbury	Cell Biologist	Litton-Bionetics
	M. Wenk	Research Scientist	Microbiological Associates
	S. Springer	Biologist	Gulf South Research Institute

* Dr. Squire resigned from NCI February 4, 1977; presently at Johns Hopkins University, Division of Comparative Medicine

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to delineate the pathogenesis and morphogenesis of pancreatic adenocarcinoma in the Syrian golden hamster, induced by N-nitroso-bis(2-hydroxypropyl)amine (BHP), a model introduced by Pour et al. (Am. J. Pathol. 76: 349, 1974). The topics of present interest are (1) the histologic appearance of the induced pancreatic neoplasms by high-resolution light (1 µm sections) and transmission electron microscopy; (2) characterization of the cell(s) of origin of the induced neoplasms and their comparison to human pancreatic cancer cells; (3) the extension of the model to include larger experimental animals, such as the guinea pig, to broaden the scope of experimental pancreatic carcinogenesis research; (4) distribution of the carcinogen in the animals, employing radiolabelled BHP, to determine the localization of the putative proximate carcinogen in the target organ(s); and (5) identification of preneoplastic markers in pancreatic carcinogenesis and their applicability as possible diagnostic tools for the early detection of malignant process.

PHS-6040
(Rev. 10-76)

Objectives: To characterize the pathogenesis and morphogenesis of pancreatic carcinoma induced by N-nitroso-bis(2-hydroxypropyl)amine (BHP; DIPN; DHPN) through the use (1) high resolution (1 μm sections) light microscopy, (2) transmission electron microscopy, and (3) quantitative microscopic autoradiography.

Methods Employed: Fifty-two test and 13 control outbred male Syrian golden hamsters, weighing 80-100 grams at the start, received BHP (250 mg/kg body wt.; 1250 mg/M^2) sc, suspended in olive oil, once weekly. They were killed in groups of 4 test and 1 control at 1 hour, 1, 2, 5, 10, 12, 15, 17, 20, 22, 26 and 31 weeks one hour after receiving an ip injection of ^{3}H-TdR (3.0 μCi/gm body wt.; specific activity 6.7 Ci/mM). Sections of pancreas were fixed and plastic embedded for high resolution (1 μm sections) light and transmission electron microscopy, and developed for quantitative light autoradiography. A second parallel study was conducted wherein distilled H_2O was substituted for the olive oil as the vehicle for the carcinogen. A third experiment sought to reproduce the findings of Pour et al. (Am. J. Pathol. 76: 349-358, 1974), who obtained a high incidence of pancreatic neoplasms in a lifetime study with outbred Syrian golden hamsters following weekly sc applications of BHP, suspended in olive oil, with a tumor latency as short as 15 weeks. A fourth parallel experiment, employing 40 test and 20 control animals per group was conducted at three dose levels (250, 125 and 25 mg/kg body wt.) of BHP dissolved in distilled H_2O, to determine the effect of a hydrophilic solvent on tumorigenesis. A fifth experiment will seek to examine the uptake and localization of carcinogen in the pancreas and other tissues by means of injection of ^{14}C-BHP or ^{3}H-BHP (at the α-position) and serial sacrifice at 5 time points of a group of 25 animals over a 72-hour period. A sixth experiment was conducted to determine the pancreatic carcinogenic potential of BHP in inbred (strain 13) guinea pigs, wherein groups of 10 male and 10 female weanling animals weighing 250-300 grams at the start received weekly sc injections of BHP in H_2O at 5 dose levels (1000, 500, 250, 125 and 63 mg/kg body wt.), with a control group of 20 animals, for a 90-day period, to establish a maximum tolerated dose for the guinea pig in anticipation of a chronic study similar in design to the hamster lifetime studies.

Major Findings: The results of the morphogenesis studies in hamsters were consistent with the hypothesis that the induced pancreatic neoplasms arise from duct epithelium, progressing from duct hyperplasia to ductal cystadenomas and papillary cystadenomas, and finally to intraductal and invasive ductal carcinomas. No acinar cell neoplasms were observed. The tumors were usually multicentric and had a predilection for the "body" and "tail" regions of the hamster pancreatic lobes. Metastases were common to local and regional lymph nodes, and occasionally to liver and lungs. Invasion of contiguous structures such as stomach, intestinal wall and spleen were commonly noted. Electron microscopy showed early non-specific damage to acinar as well as duct cells, but the acinar cells underwent regeneration and/or degeneration while the duct cells underwent neoplastic transformation. There was some evidence to suggest that a consistent and distinctive appearance of the nuclear chromatin pattern in the duct cells heralded the appearance of malignant transformation.

Other histologic patterns observed with the malignant neoplasms included cribriform proliferation, cellular anaplasia, periductal fibrosis and chronic inflammation and unusual and bizarre nuclear and nucleolar forms.

Autoradiographic data provided a good correlation between the biology and histogenesis in the hamster model system. Although a gradual increase in labelling of duct cells with time was noted, there was nonetheless a dramatic multifold increase at 22 weeks (BHP in olive oil) and 10 weeks (BHP in H_2O), corresponding precisely to the times when overt neoplasms (adenomas and carcinomas) became histologically apparent. These data were significant ($p < 0.05$) using the standard parametric two-tailed t-test as well as a non-parametric randomization test.

The results of the lifetime studies compared favorably with those of Pour et al. (op. cit.), with at least a 73% incidence of pancreatic ductal neoplasms in the (25 mg/kg. body wt.) lowest dose group and a 94% incidence in the (250 mg/kg body wt.) highest dose group (both H_2O vehicle). The incidence was 100% in the 250 mg/kg body wt.) BHP-olive oil group. An unexpected finding was a very high incidence of hepatic angiosarcomas (range 46-56%). Also encountered were significant numbers of nasal cavity (range 30-65%), pulmonary (range 30-76%), tracheal (range 7-22%), gastric (range 2-17%), colonic (range 2-5%), and other neoplasms including those of renal and hematopoietic origin. Another interesting finding was that of megalocytosis in non-neoplastic urothelial, renal and hepatic epithelial cells, which may be direct result of the carcinogen (abnormal cell division). There was no electron microscopic evidence of a viral etiology to the megalocytosis.

The effect of substituting H_2O for olive oil as the vehicle for the carcinogen was to decrease the average latency period for pancreatic tumor development from 19 to 16 experimental weeks in the comparable groups (250 mg/kg body wt.). With the H_2O vehicle, the effect of lowering the dose from 250 to 125 mg/kg body wt. resulted in increasing average survival from 23 to 34 weeks, produced about the same yield pancreatic tumors (94% and 100%, respectively), but dramatically increased the yield of tumors in other organs. At the 25 mg/kg dose level 75% of the animals developed pancreatic neoplasms.

The results of the dose-ranging study with BHP in the strain 13 guinea pigs revealed that all doses of BHP except the lowest, 62.5 mg/kg body wt., resulted in greater than a 10% body weight suppression in 13 weeks. Even at the lowest dose, weight loss was approximately 8-10%. Mortality was unaffected in those receiving the two lowest doses, but clinical signs of toxicity, weight loss, lethargy and rough hair coat were evident in all animals as well as microscopic evidence of liver pathology, including hemorrhageic necrosis micronodular cirrhosis, hepatocellular carcinoma and angiosarcoma. No evidence of pancreatic pathology, except for focal and lobular acinar cell degeneration was evident, suggesting the refractive nature of the strain 13 guinea pigs to BHP-induced pancreatic carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: Pancreatic cancer is now the 4th leading cause of cancer mortality in the United States, with an incidence that is rising alarmingly. Until the emergence of the hamster-BHP model in late 1974, no adequate animal model was available for the induction and study of pancreatic carcinoma. The project has reaffirmed the significance of this model, delineated the morphogenesis and provided a baseline for further studies by the biomedical research community in an effort to determine the etiology and search for early methods of detection and prevention of the human disease.

Proposed Course of the Project: The data will be further analyzed and prepared for publication. The guinea pig BHP protocol has been modified to continue 3 groups of surviving animals on a chronic dosing schedule (250, 125 and 62.5 mg/kg body wt./week) in an effort to document any pre-neoplastic changes in the pancreas. The carcinogen uptake and localization experiment in the hamsters await procurement of the radiolabelled BHP.

PUBLICATIONS

Levitt, M., Harris, C., Squire, R., Springer, S., Wenk, M., Mollelo, C., Thomas, D., Kingsbury, E., and Newkirk, C.: Experimental pancreatic carcinogenesis. I. Morphogenesis of pancreatic adenocarcinomas in the syrian golden hamster induced by N-nitroso-bis(2-hydroxypropyl)amine, Am. J. Pathol., In press.

Levitt, M., Harris, C., Squire, R., Springer, S., Wenk, M., Mollelo, C., and Thomas, D.: Experimental pancreatic carcinogenesis. II. Lifetime carcinogenesis studies in the syrian golden hamster with N-nitroso-bis(2-hydroxypropyl)amine, Am. J. Pathol., In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04513-02 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism of Chemical Carcinogens by Cultured Human Bronchus and Pancreatic Duct.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Harris	Head, HTSS	EXP, NCI
	H. Autrup	Staff Fellow	EXP, NCI
OTHER:	G. Stoner	Research Scientist	EXP, NCI
	J. Selkirk	Research Scientist	ORNAL, TN
	S. Yang	Visiting Associate	CB, NCI
	H. Gelboin	Chief	CB, NCI
	B. Trump	Chairman of Pathology	Univ. MD
	A. Jeffrey	Research Scientist	Inst. Cancer Res., NY
	I. Weinstein	Professor of Medicine	Inst. Cancer Res., NY
	R. Connor	Biological Statistician	DCCP, NCI

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD
Institute for Cancer Research, New York, NY

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	1.5	1.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The maintenance of human bronchus and pancreatic duct in a controlled experimental setting provides an excellent in vitro system to study the metabolism of chemical carcinogens including those found in tobacco smoke and in the environment. Cultured human bronchus can metabolically activate several classes of chemical carcinogens e.g., polynuclear aromatic hydrocarbons, acyclic and cyclic N-nitrosamines, hydrazines and mycotoxins.

The pathway of benzo[a]pyrene (BP) metabolism leading to the binding (-)-7β,8α-dihydroxy-9α,10α-epoy-7,8,9,10-tetrohydrobenzo[a]pyrene to DNA has been defined in cultured human bronchial mucosa. The major BP-DNA adduct is between the 10-position of BP and the 2-amino group of guanine. Therefore, the metabolic pathway forming the predomimant BP-DNA adduct in human bronchus is similar to that found in experimental animals in which BP is carcinogenic. A 75-fold variation among individuals in the binding of BP to DNA in cultured bronchi has been measured. Nontumorous bronchi from patients with epidermoid carcinomas of the lung bind significantly more BP to DNA than specimens from patients with either no cancer or adenocarcinoma of the lung.

PHS-6040
(Rev. 10-76)

Objectives: To determine the metabolic pathways of chemical carcinogens in human target tissues and to measure the inter-individual variation in the metabolism of carcinogens.

Methods Employed: Explant culture of human tissues; quantitative high-resolution light microscopic autoradiography; isolation of cellular macromolecules; high-pressure liquid chromatography; enzyme assays.

Major Findings: Cultured human bronchial mucosa metabolically activate procarcinogens [polynuclear aromatic hydrocarbons: 7,12-dimethylbenz[a]anthracene (DMBA), 3-methylcholanthrene (MCA), benzo[a]pyrene (BP) and dibenz[a,h]anthracene (DBA); N-nitrosamines: dimethylnitrosamine (DMN), diethylnitrosamine (DEN), N-nitrosopiperidine (NPd), N-nitrosopyrrolidine (NPy) and dinitrosopiperazine (DNP); a hydrazine: 1,2-dimethylhydrazine (1,2-DMH); and a mycotoxin: aflatoxin B_1 (AFB_1)] into forms that bind to cellular macromolecules including DNA.

The metabolism of a carcinogenic polynuclear aromatic hydrocarbon, BP, has been extensively investigated. In cultured human bronchial mucosa, activity of aryl hydrocarbon hydroxylase (AHH) was inducible 1.5 to 3-fold by benz[a]anthracene while epoxide hydratase activity was slightly reduced. BP can also induce AHH activity and thus enhance its own metabolism. Human bronchial explants release metabolites of BP into the culture medium. The metabolites extracted by organic solvents have been analyzed by high-pressure liquid chromatography (HPLC). Less than 10% of these metabolites are phenols. When compared to 9,10-diol and 7,8-diol only a small amount of 4,5-diol is formed. The majority of the metabolites are more polar compounds, e.g. tetrols and a triol which arise predominantly from (-)7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrohydrobenzo[a]-pyrene (diol epoxide I). When compared to nontumorous bronchi from patients with lung cancer, bronchial explants from patients without cancer produced more 9,10-diol and (7/8,9)-triol. In addition to the data cited above, the major BP-DNA adduct has been isolated and identified; the 10 position of BP is linked to the 2 amino group of guanine. Therefore the metabolic pathway leading to predominant BP-DNA adduct in human bronchial mucosa is similar to that found in experimental animals in which BP is carcinogenic. The removal of BP bound to DNA is more rapid than its removal from protein. The repair (loss) of BP-DNA adducts is currently being studied.

An individual's risk of developing cancer may in part depend on the balance between activation and deactivation of procarcinogens. Initial studies have focused on the formation of BP-DNA adducts as a resultant of these competing metabolic processes. The binding of BP to DNA in cultured human bronchus was measured in specimens from 57 patients. A 75-fold variation in binding levels was observed which is similar in magnitude to that found in pharmacogenetic studies of drug metabolism. When compared to patients without lung cancer and to those with adenocarcinoma of the lung, nontumorous specimens from patients with epidermoid lung cancer had significantly higher levels of BP bound to DNA. Binding of BP to DNA was inhibited by 7,8-benzoflavone and anti-oxidants (butylated hydroxytoluene, disulfiram and vitamin E) but not altered by either

nicotine or -retinyl acetate. Binding of DMBA to DNA in pancreatic duct was consistently several-fold lower than binding levels in human bronchus.

Metabolism of carcinogenic N-nitrosamines was studied in normal-appearing bronchial specimens obtained from four patients. Explants of bronchi were cultured in a chemically-defined medium for seven days. N-Nitrosamines [DMN, DEN, DNP, NPy, and NPd] labeled with ^{14}C were each then added to 100 µM for 24 hr. Measurable CO_2 was formed by bronchial explants from: 1) DMN, DEN and NPy in all four patients; 2) DNP in three of four patients; and 3) NPd in only one of four patients. In all bronchial specimens, binding of these N-nitrosamines and/or their metabolites to bronchial mucosal DNA and protein was found. Binding levels were higher to protein than to DNA. DMN methylated DNA at both O-6 and N-7 positions of guanine. Binding levels of DNP were as high as those with the two acyclic N-nitrosamines, DMN and DEN, while binding levels of NPy and NPd were lower. Human bronchus can metabolize acyclic and cyclic N-nitrosamines that are found in the environment and in tobacco smoke.

Both 1,2-DMH and AFB_1 are metabolized by cultured human bronchi. Binding of these carcinogens to macromolecules was dependent on dose. 1,2-DMH methylated guanine in DNA at both O-6 and N-7 positions.

Significance to Biomedical Research and the Program of the Institute: It would appear that methodologies developed for and utilized in studies of carcinogenesis in experimental animals and cell cultures can be successfully extended to similar investigations in human cells and tissues. These investigations should eventually aid in identifying both chemical carcinogens and host factors determining susceptibility. Finally, these studies provide a much needed link between studies in experimental animals and man.

Proposed Course of the Project: Identify genetic and exogenous factors that alter the metabolism of environmental procarcinogens. Develop ultramicroassays of carcinogen metabolism so that metabolism can be studied in biopsy specimens. Determine the metabolic pathways of N-nitrosopyrrolidine.

Publications

Harris, C., Autrup, H., Connor, R., Barrett, L., McDowell, E., Trump, B.: Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi. Science 194: 1067-1069, 1976.

Weinstein, I. B., Jeffrey, A., Jennette, K., Blobstein, S., Harvey, R., Harris, C., Autrup, H., Kasai, H., Nakanishi, K.: Benzo[a]pyrene diol-epoxide as intermediates in nucleic acid binding in vitro and in vivo. Science 193: 592-595, 1976.

Yang, S. F., Gelboin, H. V., Trump, B. F., Autrup, H., Harris, C. C.: Metabolic activation of DNA binding of benzo[a]pyrene in cultured human bronchus. Cancer Res. 37: 1210-1215, 1977.

Harris, C., Autrup, H., van Haaften, C., Connor, R., Frank, A., Barrett, L., McDowell, E., Trump, B.: Inhibition of benzo[a]pyrene binding to DNA in cultured Human Bronchi. In Nieburgs, H. (Ed.), Proc. 3rd Int. Symposium Cancer Detection and Prevention. Excerpta Medica, Amsterdam, In press.

Harris, C. C., Autrup, H. M., Stoner, G. D., Barrett, L. A., McDowell, E. M., Trump, B. F.: Binding of dimethylnitrosamine and 1,2-dimethylhydrazine to macromolecules in cultured human bronchi. Cancer Res. In press.

Harris, C., Autrup, H. and Stoner, G.: Metabolism of benzo[a]pyrene by cultured human tissues and cells. In Ts'o, P.O.P. and Gelboin, H. V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment, Academic Press, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04548-05 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Registry of Experimental Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Harold L. Stewart	NIH Scientist Emeritus	EXP NCI
OTHER:	Thelma B. Dunn	Consultant	EXP NCI
	Margaret K. Deringer	Biologist	EXP NCI
	(Margaret D. Barrett)		

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Tumor Pathology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The objectives of the Registry of Experimental Cancers are 1) the storage and retrieval of pathologic material and data on cancers and other lesions of laboratory animals (primarily rodents), and 2) the use of such information for research and educational purposes.
2. The Registry has acquired a total of 969 (300 since the 1976 report) single or group accessions from investigators outside of NCI. Approximately 28,000 (5000 since the 1976 report) records have been prepared for coding and coded.
3. Sixty-three investigators have come to the Registry for consultations, on single or multiple visits.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: 1) The storage and retrieval of pathologic material and data on cancers and other lesions of laboratory animals (primarily rodents), and 2) the use of such for research and educational purposes.

Methods Employed: The methods employed in the work of the Registry involve the selection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens; and illustrations in the form of lantern slides, gross photographs, and photomicrographs in black and white and in color. The work of the Registry also includes the collection of records of experiments, reprints and references on this material. The Registry of Experimental Cancers possesses a large collection of spontaneous and induced cancers and other lesions. The pertinent information on the collection is being indexed. Many of the data have been prepared for and entered into the computer. The Registry accesses material from investigators at NCI, other institutes of NIH, other governmental agencies, industrial laboratories, and universities here and abroad. A total of 969 single or group accessions from investigators outside of NIH have been processed since 1971. The Registry is preparing Study Sets of slides, with explanatory material, relating to particular areas of cancer in rodents.

The Registry has Study Sets of slides on "Hematopoietic and Lymphoreticular Neoplasms", "Induced and Spontaneous Tumors of Small Intestine and Peritoneum in Mice", "Tumors and Nonneoplastic Proliferative Lesions of the Lungs of Mice", and "Induced Tumors of the Liver in Rats", which are loaned with descriptive material, to investigators who request them. Seventeen loans up to two months have been made this year.

Investigators come to the Registry for consultation. There have been single or multiple consultations with 63 individuals since May 1976.

Major Findings: The function (outlined in objectives) of the Registry in the wider field of cancer research and more particularly in the Carcinogenesis Program are such that there will be no major findings to report.

Significance to Biomedical Research and the Program of the Institute: The availability of the wealth of material possessed by the Registry advances the knowledge of spontaneous and induced disease processes in animals.

The existence of the Registry will contribute to the standardization of nomenclature of cancers and other lesions in laboratory rodents. Slides and protocols from the Registry are used to illustrate and describe lesions discussed at the weekly slide conferences.

The members of the Registry serve as consultants in the monitoring of pathology from laboratories under the Bioassay Program contracts.

The NAS, NRC, ILAR Subcommittee on Rat Liver Tumors Committee on Histological Classification of Laboratory Animal Tumors has held all of its meetings, 3 to date, at the Registry. The subcommittee is using the Registry's Study Set of "Induced Tumors of the Liver in Rats" as a basis for the histologic typing of liver tumors in this species. The histologic classification and typing of tumors in laboratory animals is calculated to promote uniformity of diagnoses from one laboratory to another in this country. This should be of inestimable value to the Bioassay Program.

The Director-General of the World Health Organization designated the Registry of Experimental Cancers as the WHO Collaborating Centre for Reference on Tumours of Laboratory Animals on 26 October 1976. This is the only such registry in the world to be so designated by the WHO. This will expand communications between scientists of this country and those of other countries, now numbering 153, which are members of WHO.

Proposed Course of the Project: The Registry will continue and expand all of its activities (already set forth in this report).

Publications

Stewart, H. L.: Some observations on comparative vertebrate and invertebrate pathology: a summary discussion of the workshop. Marine Fisheries Rev. 38:46-48, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04567-04 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogenesis Studies in Human Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	H. Autrup	Staff Fellow	EXP, NCI
	I. Hsu	Research Scientist	EXP, NCI
	M. Levitt	Research Pathologist	EXP, NCI
	E. McDowell	Assoc. Professor of Pathology	Univ. MD
	P. Schafter	Professor of Surgery	WVAH
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	M. Valerio	Princ. Invest., Litton Bionetics	Kensington, MD

COOPERATING UNITS (if any)
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University of Maryland, School of Medicine, Baltimore, MD
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LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	3.5	3.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Model systems for studying carcinogenesis in human target tissues (bronchus, pancreatic duct, breast and colon) are being developed to link investigations using experimental animals with human cancer. Metabolism of chemical carcinogens is being studied in human tissues maintained in these model systems. For example, a marked variation among individuals in their capacity to metabolize carcinogenic polynuclear aromatic hydrocarbons has been observed. This inter-individual variation may, in part, be due to genetic factors. Cultured human bronchi convert chemical carcinogens, e.g., benzo[a]pyrene, into metabolites that are mutagens in mammalian cells (V-79 cells). This mutagenesis assay is an important new screening test for chemical carcinogens. Chemical and physical carcinogens cause basal cell hyperplasia and atypical squamous metaplasia in cultured and xenotransplanted human bronchi. Model systems using human tissues should be useful in several areas of cancer research including: 1) identifying environmental carcinogens, their metabolic pathways and host factors determining susceptibility; and 2) testing new methods of prophylatic intervention in populations at high risk of developing cancer.

PHS-6040
(Rev. 10-76)

Objectives: To develop experimental systems to study carcinogenesis in human target tissues under controlled conditions. Such systems involve 3 major facets: 1) collection of viable human tissues; 2) in vitro maintenance of epithelial cells in these tissues in cell and organ cultures; and 3) xenotransplantation of human tissues into immunodeficient animals.

Methods Employed: Human tissues are obtained at either surgery or "immediate" autopsy. The tissue is maintained in culture and xenotransplanted into athymic nude mice. These tissues are exposed to chemical carcinogens in vitro and/or as a xenograft. The morphological lesions caused by carcinogens are examined by light and electron microscopy. Binding of carcinogens to macromolecules in epithelial cells is measured by biochemical and autoradiographic methods, which were previously developed, in part, in studies of animal models.

Major Findings: The methodology for each of the three major technical facets of this program (collection of tissues, explant culture and xenotransplantation) have been refined. The maintenance of human tissue (bronchus, colon and pancreatic duct) in controlled experimental settings has allowed a series of interlocking studies including: 1) the metabolism of chemical carcinogens by human tissues and cells; 2) mutagenesis in mammalian cells (V-79 Chinese hamster cells) mediated by human tissues; and 3) induction of epithelial lesions by chemical and physical carcinogens. Significant progress has been made in each of the above areas of investigation. Several classes of chemical carcinogens including many of those found in tobacco smoke as well as the environment are metabolically activated into forms that bind to cellular DNA. A marked inter-individual variation in levels of carcinogen-DNA adducts has been found. For example, the variation in binding levels of benzo[a]pyrene to DNA were 75-fold for bronchus (57 patients) and 60-fold for colon (30 patients). The relationship between formation of carcinogen-DNA adducts and individual's oncogenic susceptibility to the carcinogen is as yet not known. These biochemical studies of carcinogen binding to DNA are being correlated with biological studies, e.g., human tissue-mediated mutagenesis. Both benzo-[a]pyrene and its proximate carcinogen, 7,8-diol, are enzymatically activated into metabolites which cause mutations in V-79 cells. While direct proof is as yet lacking it seems prudent to consider chemicals as being carcinogenic in humans if they 1) are carcinogenic in experimental animals; 2) form adducts with DNA in human epithelial cells; and 3) are converted by human tissues and cells to mutagens in experimental mutagenesis assays.

Studies in progress suggest that carcinogens cause basal cell hyperplasia and atypical squamous metaplasia in cultured and in xenotransplanted human bronchial epithelium. The biological fate of these preneoplastic lesions is being determined.

The various facets of these studies are described in detail (Intramural Research Project Numbers Z01-CP-04485-01-EXP, Z01-CP-04486-01-EXP, Z01-CP-04487-01-EXP, Z01-CP-04488-01-EXP, Z01-CP-04489-01-EXP, Z01-CP-04513-02-EXP, and Z01-CP-04517-01-EXP. These investigations are conducted in collaboration with NIH contractors, University of Maryland (N01-CP-43237), Litton Bionetics (N01-CP-43274) and Washington Veterans Administration Hospital (Y01-CP-60204).

Surgical and autopsy specimens are provided to NCI and to Litton-Bionetics by Washington Veterans Administration Hospital and by University of Maryland. Methods to culture explants of human tissues were developed in parallel at the University of Maryland and the NCI. Methods to culture human epithelial cells are being established at the NCI. Investigations concerning the metabolism of chemical carcinogens and the development of the human tissue mediated mutagenesis assay were conducted primarily at the NCI. Cultured tissues are exposed to carcinogens at the NCI and at the University of Maryland. Human tissues are implanted into athymic nude mice at Litton-Bionetics.

Significance to Biomedical Research and the Program of the Institute: The extrapolation of carcinogenesis data from studies utilizing experimental animals in man presents several complex problems. One approach to provide a link between these experimental data and human cancer is the development of an experimental system to study carcinogenesis in important human target tissues. Such a system could aid in the identification of individuals who are highly susceptible to environmental carcinogens, and methods of intervention could be tested in this experimental system prior to clinical trials in man.

Proposed Course of Project: Attempts are continually being made to improve each interlocking facet of this program. Studies have been initiated to develop cell cultures derived from human bronchial and pancreatic epithelial cells. These cultured cells will be characterized by both morphological and biochemical methods. Attempts to transform the epithelial cells with chemical carcinogens will be made. Correlations will be made among the formation of carcinogen-DNA adducts in human tissues, mutations in V-79 cells mediated by human tissues and morphological lesions in cultured human epithelia caused by chemical carcinogens.

Publications

Harris, C.: Chemical carcinogenesis and experimental models using human tissues. Beit. Pathol. 158: 389-404, 1976.

Harris, C.: The carcinogenicity of anticancer drugs: a hazard in man. Cancer 37: 1014-1023, 1976.

McDowell, E., Barrett, L., Harris, C., and Trump, B.: Abnormal cilia in human bronchial epithelium. Arch. Pathol. 100: 429-436, 1976.

Harris, C., Cohen, M., Connor, R., Primack, A., Saccomanno, G., and Talamo, R.: Serum alpha$_1$-antitrypsin in patients with lung cancer or abnormal sputum cytology. Cancer 38: 1655-1657, 1976.

Harris, C., Autrup, H., Stoner, G., and Trump, B.: Model systems using human lung for carcinogenesis studies. In Harris, C. (Ed.): Pathogenesis and Therapy of Lung Cancer. New York, M. Dekker Inc., 1977, In press.

Harris, C.: Respiratory carcinogenesis. In Straus, M. (Ed.): Lung Cancer: Clinical Diagnosis and Treatment. Grune and Stratton, 1976, In press.

Harris, C. (Ed.): Pathogenesis and Therapy of Lung Cancer. New York, M. Dekker, Inc., 1977, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04687-07 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transplacental and Neonatal Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P. Roller	Research Chemist	CMT, NCI
	J. Ward	Vet. Med. Officer	EXP, NCI
	W. T. London	Head, EXP	NINCDS, NIH
	M. Wenk	Project Director	Microbiological Associates, Inc.
	P. Kleihues	Professor	University of Freiburg/Br., Germany

COOPERATING UNITS (if any)

NINCDS

LAB/BRANCH
Experimental Pathology Branch, Carcinogenesis Program

SECTION
Perinatal Carcinogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	5.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The roles of metabolism, immune processes, DNA repair, and other factors related to susceptibility to transplacental carcinogenesis in rodents and primates are investigated, with emphasis on nitrosamine and aromatic hydrocarbon carcinogens and utilizing both whole animal and in vitro culture techniques.

PHS-6040
(Rev. 10-76)

Objectives: To develop experimental animal models for human cancer of childhood, and to study the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, as well as the roles of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy.

Methods Employed: Carcinogenic organic compounds are synthesized where necessary; organic polymers are obtained commercially and characterized spectroscopically, and purified where necessary. Animals of various species and strains, including non-human primates, are exposed to these compounds at different times during pre- and postnatal life, and the carcinogenic response in various organs is evaluated histologically. Short and long-term cell and organ culture techniques are used where necessary to explore the role of host defense mechanisms in resistance to carcinogenesis, and the role of differentiation processes in determining the histological features and biological behavior of transplacentally induced tumors. Surgical procedures, including skin grafting, adult thymectomy, etc., are used in evaluating antitumor defense mechanisms of the intact animal, and organic and biological chemical techniques are employed to determine the effects of modifications of chemical carcinogen structure and of metabolic factors on the response of host tissues to carcinogens. Standard biochemical procedures (alkaline sucrose gradient ultracentrifugation; in vitro enzyme-mediated protein synthesis from DNA templates; colorimetric assays of enzyme activity) and autoradiography are employed to investigate the fidelity and ontogeny of DNA repair capability in the developing fetus subsequent to carcinogen-induced damage.

Major Findings: Methyl(acetoxymethyl)nitrosamine (DMN-OAc), an ester of the presumed carcinogenic metabolite of dimethylnitrosamine, was previously reported preferentially to induce epithelial tumors of the intestines following intraperitoneal administration to weanling rats, and brain and kidney tumors in the offspring of rats injected intravenously during pregnancy. Studies on the pathogenesis and natural history of the intestinal tumors induced by DMN-OAc indicate that the biological behavior of adenocarcinomas of the small intestine in the rat, particularly their tendency to metastasize, may be related to the magnitude and fractionation of the dose of carcinogen administered. This phenomenon is under further study. The route of administration has been found to be crucial in determining the sites of carcinogenesis by DMN-OAc; SC injection give rise principally to local sarcomas, PO treatments to gastric carcinomas, and IV injections to lung and zymbal's gland tumors. The specificity for the intestinal mucosa of DMN-OAc injected IP is thus most likely a pharmacodynamic rather than a metabolic phenomenon.

Repeated IV injections of ethylnitrosourea (ENU) have resulted in the development of malignant tumors of the blood vasculature, bone, lymphoreticular tissue, ovaries and uterus of 8 of 13 patas monkeys (Erythrocebus patas) given subtoxic injections every 14 days for 2 years, starting after weaning but before attainment of sexual maturity. Transplacental exposure to ENU has induced 5 tumors in 4 monkeys before the age of 1 year. These included 2 hemangiosarcomas, 1 hepatocellular tumor, 1 leiomyosarcoma of the lung, and a squamous papilloma of the skin. One of the vascular tumors was present at birth. These

4 monkeys were among the products of 20 pregnancies in which exposure to ENU was begun during the first month of the 165 day gestation period, usually at day 30. A comparable number of animals whose in utero exposure to ENU was of comparable magnitude, but begun during the second month, have been observed for as long as 3 years without detection of any neoplasm. The period of greatest vulnerability to the direct-acting carcinogen ENU appears to be early in gestation in this primate species, and is roughly coincident with, rather than subsequent to, the period of greatest teratogenic susceptibility.

Cell cultures of developing nervous tissue have been established to explore the resistance of rat CNS tissue to carcinogens prior to day 12 of gestation and the failure of medulloblastomas to arise from surviving cells of the external granular layer of the fetal rat cerebellum following overt damage by DMBA. These neurectodermal cells rapidly acquire neoplastic properties in vitro even in the absence of exposure to carcinogens; these properties include ability to grow in soft agar media and to form invasive tumors in athymic mice. Spontaneous transformation appears less of a problem with patas monkey cells, and emphasis is being shifted to the primate species.

A number of organ and cell culture techniques have been developed for fetal organs. Metanephric rudiments from both rat and mouse can be maintained for brief periods in serum-free media, and incorporate tritiated thymidine in quantities sufficient for autoradiography. Dispersed cell culture of fetal and adult rat kidney has been established, and histochemical markers for both fetal and adult tubules have beeen demonstrated, including 5'-nucleotidase, glucose-6-phosphatase, and γ-glutamyl transpeptidase. As yet, no enzyme has been found exclusively or primarily in fetal kidney. A continuing effort to manipulate morphology and differentiation of normal and neoplastic renal tissue through media additives is under way. Mouse kidneys develop purely epithelial, well-differentiated tumors of adult type in response to transplacental carcinogens, while rat kidneys generate adult epithelial, neophroblastic, and undifferentiated (blastemal cell) tumors; the ability of rat and mouse renal tumor cells to respond to differentiation signals and the influence of the latter on their morphology, growth, and biologic behavior are being investigated.

Studies on the capacity of fetal organs to repair damage to DNA resulting from exposure to carcinogens have been initiated utilizing alkaline sucrose gradient ultracentrifugation for detecting DNA strand breakage and autoradiography for confirming unscheduled DNA synthesis. A preliminary study on the fidelity of DNA repair of lesions inflicted by different classes of mutagens and carcinogens has been begun, utilizing a bacteriophage/host bacterium repair system and an in vitro system for β-galactosidase synthesis from the phage DNA to evaluate fidelity of repair to the β-galactosidase structural gene. Both projects are in a preliminary stage and no data are yet reportable.

Significance to Biomedical Research and the Program of the Institute:
Research for animal models of human childhood neoplasms should provide an insight into the types of causative agents and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fetal neoplasms before attaining reproductive age, yet the incidence of tumors in childhood is relatively constant. Epidemiological studies have pointed to the occurrence of childhood neoplasms in association with certain types of congenital malformation which are non-inherited, and suggest that environmental agents, alone or in combination, may play a role in the induction of such neoplasms. The ability to induce medulloblastomas chemically in mice allows studies on the nature and inducibility of these tumors to proceed. The fact that such tumors are not induced in the closely related rat by identical treatment may provide an experimental approach to identifying factors which are important for their development.

Most tumors induced transplacentally in rodents are of adult types and appear during adult life in individuals exposed in utero, resembling human experience with diethylstilbestrol. The ENU studies in patas monkeys will provide experimental data indicating whether adult or pediatric tumor types are more likely to develop in at least one species of primate in response to carcinogenic exposure in utero, and thus whether chemical carcinogens are likely to be involved in the genesis of pediatric or adult types of tumors in man.

Carcinomas of the gastrointestinal tract and pancreas comprise the largest source of cancer morbidity in the adult population of the United States. Existing "intestine-selective" carcinogens are much less specific than is desirable. The high specificity of methyl(acetoxymethyl)nitrosamine for intestinal mucosa provides a new carcinogen well suited to studies of physiological and biochemical factors significant in development of intestinal carcinoma.

Studies in rodents have shown that a fetus may be up to 4 orders of magnitude more susceptible to carcinogens than an adult of the same species, strain and sex, yet the precise reason for this enhanced vulnerability are not clearly understood. Moreover, in rodents, many tumors that develop as a consequence of exposure of incompletely differentiated cells to chemical carcinogens are morphologically identical to those inducible in adults, suggesting that the fundamental damage inflicted on such cells does not preclude subsequent programmed differentiation. The possibility that fetal cells may be deficient in activity or fidelity of DNA repair processes is a major definable physiologic function which might contribute to enhanced fetal susceptibility to these agents. The fact that differentiation overrides neoplastic transformation in a given organ system (e.g., kidney) of certain species, but not others, provides a route to exploration of the basic nature of cellular differentiation in the control of neoplastic growth.

Proposed Course of the Project: Direct and transplacental carcinogenesis by DMN-OAc and its ethyl homolog, DEN-OAc, will be explored further to define the effects of different doses and routes of administration on different strains of rats, mice, and hamsters. The metabolism and pharmacodynamics of distribution and excretion, especially the role of bile, will be studied using DMN-OAc-^{14}C.

Studies of mouse and rat neurogenic tumors and their precursors will be continued with emphasis on (1) medulloblastomas; (2) the establishment of cultures of possible medulloblastoma precursor cells from DMBA-treated rats; (3) identification of differentiated biochemical functions to confirm or exclude the hypothetical neuroectodermal origin of several classes of spontaneous and chemically inducible tumors of rats and mice; (4) the nature of the facile and rapid spontaneous neoplastic transformation of untreated rat neurectodermal cells when explanted to monolayer cell culture.

Patas monkeys exposed to ENU or DEN either transplacentally or directly (after weaning) should continue to be observed for the development of tumors. In vivo studies should emphasize further refinement of definition of periods of maximal prenatal susceptibility to direct-acting vs. enzyme-activated transplacental carcinogens. Pharmacodynamic studies will be continued to further compared maternal-fetal distribution and tissue/organ localization of ENU-^{14}C in this species in comparison to rats and mice. Comparative DNA repair capacities of primate and rodent tissues after ENU treatment will be studied further, and extended to modalities of repair other than O-6 alkylation product elimination.

Rat renal "blastemal cell" tumors will continue to be studied in organ culture to determine whether or not the morphologically mesenchymal tumors can be induced to form characteristic renal tubules and glomeruli or the enzymes characteristic of renal epithelium, thus confirming the relation of such neoplasms to nephroblastomas. Once the model is established, it sould be used to determine the effects of manipulations of physiological parameters, including hormonal balance and immunological responsiveness, on the genesis and behavior of these tumors.

The possible role of DNA repair processes in modifying susceptibility of differentiating tissues to carcinogens should be actively investigated by both density gradient ultracentrifugation and autoradiographic techniques. The separate question of fidelity of repair of lesions in DNA inflicted by different classes of mutagenic and carcinogenic agents should first be explored in a simple, well-defined microbial system, with subsequent extension to mammalian systems if the gene product analysis approach appears promising.

Publications

Rice, J. M.: Environmental Factors: Chemicals. In Brent, R. L. and Harris, M. (Eds.): Prevention of Embryonic, Fetal, and Perinatal Disease. DHEW Publ. No. (NIH) 76-853. Wash., D.C., U.S. Govt. Print. Off., 1976, 163-177 pp.

Levy, B., London, W., Fucillo, D. A., Baron, S., and Rice, J.: Prophylactic control of simian hemorrhagic fever in monkeys by an interferon inducer, polyrioinosinic polyribocytidylic acid-poly-L-lysine. J. Infect. Dis. 133 Suppl: A256-A259, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04798-07 EXP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Role of Vitamin A in Control of Epithelial Differentiation and Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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Experimental Pathology Branch, Carcinogenesis Program

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The scope of this project is to understand the molecular mode of action of vitamin A in controlling epithelial differentiation and growth. The following studies have been conducted in the past year.

Phosphorylated derivatives of retinol and retinoic acid (retinoids) have been synthesized and studied for their ability to function as acceptors of D-mannose from guanosine diphosphate mannose in a reaction catalyzed by rat liver membranes. It was found that the phosphate esters of retinol and its derivatives were efficient acceptors of D-mannosyl residues. The mixed anhydrides of all-trans-β-retinoic acid, 13-cis-retinoic acid and phosphoric acid did not function as mannosyl acceptors. Mannosyl retinyl phosphate was found to donate D-mannose to specific endogenous glycoproteins of the rat liver membranes. These glycoproteins were susceptible to pronase, yielding glycopeptides.

Another glycosyl derivative of the phosphorylated vitamin, galactosyl retinyl phosphate, was isolated from cultured epidermal cells.

Objectives: To study the mode of action of vitamin A in maintenance of normal epithelial cell differentiation and growth in mammals.

Methods Employed: (a) Chemical synthesis of phosphorylated retinoids: Methods for the chemical synthesis of phosphorylated retinoids include the use of di-triethylamine phosphate as the phosphorylating agent in a mixture of aceto-nitrile and trichloroacetonitrile; the reaction is conducted in the dark and the phosphorylated retinoid is isolated and purified by chromatography on columns of DEAE-cellulose. (b) Selective extraction of retinyl phosphate and its glycosylated derivatives from membranes from different types of cells: Retinyl phosphate and its glycosyl derivatives are extracted from the membranes with 99% methanol. This solvent leaves dolichyl phosphate and its glycosyl derivatives unextracted in the denatured membranes. (c) A solvent extraction procedure has also been developed to separate glycosyl derivatives of retinyl phosphate from glycosyl derivatives of dolichyl phosphate; the latter compounds remain in the lower phase of the Folch extraction procedure, whereas retinyl phosphate and its derivatives can be extracted in the upper phase.

Major Findings: The following phosphorylated retinoids were prepared as described: all-trans-β-retinyl phosphate; all-trans-9-(4-methoxy-2,3,6-tri-methylphenyl)-3,7-dimethyl-2,4,6,8 nonatetraene-1-yl phosphate; all-trans- -retinoyl phosphate; 13-cis-β-retinoyl phosphate. These compounds were tested as acceptors of D-mannose from guanosine diphosphate mannose in a reaction catalyzed by rat liver membranes. The phosphate esters all functioned as acceptors of mannose to give a product chromatographically indistinguishable from endogenous mannosyl retinyl phosphate. The mixed anhydrides, however, did not function as mannosyl acceptors. Neither class of compounds had any effect on the biosynthesis of dolichyl mannosyl phosphate.

Rat liver membranes did not catalyze the transfer of D-galactose from uridine-diphosphate galactose to retinyl phosphate, even at the concentration of retinyl phosphate (0.73 mM) which stimulated the synthesis of mannosyl retinyl phosphate by more than 50 fold.

Membranes from cultured mouse epidermal cells catalyzed the synthesis of galactosyl retinyl phosphate, while rat liver membranes did not. Thus, there appears to be specificity for the particular monosaccharide accepted and possibly transferred by retinyl phosphate in different tissues.

A variety of mammalian tissues was tested for the GDP-man:retinyl phosphate mannosyl transferase. The enzyme appeared to be ubiquitous. In contrast, the UDP-galactose:retinyl phosphate galactosyl transferase appeared to be located exclusively in the epidermis.

Transfer of D-mannosyl residues from mannosyl retinyl phosphate (MRP) to endo-genous acceptors of rat liver membranes was also investigated. MRP was found to be an effective donor of mannose to glycoprotein acceptors. Such

donor activity was lost after exposure to ultraviolet light for three periods of one minute each, in ice. The glycoprotein acceptor was digested by pronase and it was cleaved by treatment with alkali, a characteristic of O-glycosidically-linked glycoproteins.

Significance to Biomedical Research and The Program of the Institute. This project contributes to the identification of key control mechanisms in the differentiation of epithelial cells; it is a part of the experimental pathology program addressed to the development of mechanisms of inhibition and control of epithelial carcinogenesis. The control of mucous-squamous cell differentiation and membrane growth is of value in understanding the mechanism(s) of carcinogenesis in epithelial tissues in general and in developing possible preventive measures. The vitamin controls epithelial cell differentiation and its absence leads to hyperplasia of basal cells, squamous metaplasia and keratinization, a sequence of events similar to those taking place in several epithelia after exposure to carcinogens.

Proposed Course of Project: 1) To characterize further the specific membrane glycoproteins which function as acceptors of mannose from mannosyl retinyl phosphate and to investigate their localization and function in mammalian membranes.

2) To study the role of retinoic acid or its metabolites in glycoprotein synthesis.

Publications

Frot-Coutaz, J. P., Silverman-Jones, C. S., and De Luca, L. M.: Isolation, characterization and biological activity of retinyl phosphate from hamster intestinal epithelium. J. Lipid Res. 17: 220-230, 1976.

Silverman-Jones, C. S., Frot-Coutaz, J. P., and De Luca, L. M.: Separation of mannosyl retinyl phosphate from dolichyl mannosyl phosphate by solvent extraction. Anal. Biochem. 75: 664-667, 1976.

Frot-Coutaz, J. P. and De Luca, L. M.: Chemical synthesis of all-trans-β-retinoyl phosphate. Biochem. J. 159: 799-801, 1976.

De Luca, L. M.: Review on mode of action of vitamin A in epithelial tissues. Vitamins (Japan) 50: 345-349, 1976.

De Luca, L. M.: Epithelial membranes and vitamin A. In Jamieson, G. A. and Robinson, D. M. (Eds.): Mammalian Cell Membranes. London, Butterworths, 1976, Vol. III, pp. 231-249.

De Luca, L. M., Frot-Coutaz, J. P., Silverman-Jones, C. S., and Roller, P. R.: Chemical synthesis of phosphorylated retinoids: their mannosyl acceptors activity in rat liver membranes. J. Biol. Chem. 252: 2574-2579, 1977.

Sundaresan, P. R. and De Luca, L. M.: Vitamin A contents of rat intestinal epithelium and jejunal mucinous adenocarcinoma. J. Natl. Cancer Inst. 1977, In press.

De Luca, L. M.: The molecular involvement of vitamin A in glycosyl transfer reactions. In Vitamins and Hormones. New York, Academic Press, 1977, In press.

De Luca, L. M.: Vitamin A. In De Luca, H. F. (Ed.): Fat-Soluble Vitamins. New York, Plenum, 1977, In press.

SUMMARY REPORT

LUNG CANCER BRANCH

July 1, 1976 through September 30, 1977

"Plans, develops and implements a research program on the pathogenesis and prevention of lung cancer involving (1) identification and study of agents and mechanisms involved in the induction and development of lung cancer in man; (2) correlation of findings in human and animal studies in order to define relevance of animal models for study of human lung cancer; and (3) identification and study of agents and mechanisms capable of preventing the development of lung cancer. All these studies are pursued in the whole organism, in isolated cellular systems involving organ and tissue culture, and at the molecular level, in animals and man."

Current studies in the Lung Cancer Branch include investigations on the carcinogenic effects of different chemicals on the respiratory epithelium in the whole animal, the use of short-term and long-term organ culture systems to study the effects of carcinogens and anti-carcinogens on respiratory and bladder epithelium, and investigation of the cellular and molecular mechanism of action of the anti-carcinogenic family of compounds known as retinoids, comprised of vitamin A and its synthetic analogs. Studies are pursued within the Branch in a manner that allows correlation of findings at the molecular, cellular, and whole animal level. The following is summary of the investigations within the Branch:

Lung Cancer Pathogenesis Section - *"(1) Develops and evaluates animal models for study of induction and prevention of lung cancer, and the role of various chemicals in inducing or preventing lung cancer. Studies involve the administration of carcinogenic and anti-carcinogenic agents to experimental animals and the evaluation of their effects on the development of lung cancer; (2) develops and evaluates organ culture and tissue culture methods for study of induction and prevention of lung cancer. Studies measure the effects of carcinogenic and anti-carcinogenic agents on the cell population which is directly involved in the development of lung cancer; and (3) studies molecular processes involved in control of cellular differentiation in respiratory epithelium as related to the induction and prevention of lung cancer. Particular emphasis is placed on the biochemical mechanisms whereby selected compounds inhibit or prevent the development of lung cancer."*

(1) Animal Studies of Respiratory Carcinogenesis - A new technique for study of respiratory carcinogenesis, employing a special cannula, has been developed very recently at the Oak Ridge National Laboratory. This cannula allows direct application of soluble carcinogens to a particular segment of the respiratory tract of an experimental animal. This particular area then can be observed for the development of preneoplastic and neoplastic lesions,

and factors that enhance or prevent the development of respiratory cancer can be intensively studied. The advantage of the new method is that one can study the pathogenesis of respiratory cancer at a defined locus in the respiratory tract, in an otherwise healthy animal that does not have multiple lesions throughout both lungs. Within the past year, extensive studies with this special cannula have been pursued in the Lung Cancer Branch. Hamsters have been exposed to various levels of nitrosomethylurea with this cannula, and squamous cell cancer has been induced. During the course of carcinogenesis, cytology specimens have been obtained without sacrificing the hamsters and have been evaluated with exfoliative cytological techniques. This will provide a useful method for further studies of markers of premalignant states.

Since a better understanding of premalignant states in the respiratory epithelium is of vital importance to understanding the development of lung cancer, the Branch is attempting to develop new methods to evaluate and quantitate preneoplastic epithelial lesions. The efforts involve the use of a newly developed image-analyzing microscope, the Bausch and Lomb "Omnicon". It has been determined that the Omnicon can measure the degree of cellular hyperplasia in an epithelium by quantitating the number of nuclei, cross-sectional area of nuclei, and total epithelial area. This instrument has also been used during the past year to measure nuclear/cytoplasmic ratios in respiratory epithelia undergoing carcinogenesis. Although this technology of image analysis is still in its infancy, it would appear to have great promise for objective evaluation of preneoplastic states.

(2) Organ Culture Studies on the Isolated Tracheobronchial Epithelium of the Hamster - Excellent progress has been made in this area during the past 12 months. Extensive use is now being made of the system which has been developed in the Lung Cancer Branch for long term organ culture of hamster trachea. A chemically defined medium, supplemented with insulin and hydrocortisone, is being used to give excellent long-term in vitro survival of hamster tracheobronchial epithelium, for 2 weeks or more. Using this system, it has been demonstrated that numerous synthetic analogs of vitamin A (retinoids) can reverse keratinizing, squamous metaplastic lesions of hamster tracheal epithelium. In addition to the further confirmation of previous findings that analogs with modifications in the ring portion of the molecule are highly active biologically, it has been found that the polar, terminal portion of the molecule can also be modified with substantial retention of activity. These new synthetic molecules include retinylidene acetone, axerophthene, retinoic acid 2-hydroxyethyl amide, and retinyl butyl ether derivatives of vitamin A, which appear to be substantially less toxic than natural forms of vitamin A. The reduction in toxicity offers major advantages in terms of development of a synthetic analog which would eventually be useful for preventiton of epithelial cancer in man. These analogs are also being evaluated for in vivo effects on specific target epithelia, such as respiratory epithelium, bladder epithelium, or mammary epithelium. The scientific collaboration of Hoffmann-La Roche, Inc., Johnson & Johnson, and BASF Aktiengesellschaft has been invaluable in the performance of the above studies.

(3) Studies on Cell Biology and Carcinogenesis in Other Epithelia - Since bladder epithelium shows many biological similarities to respiratory epithelium (including a relatively high incidence of bladder cancer in people who smoke heavily), studies have been continued during the past year on organ culture of bladder epithelium. Optimal culture conditions for bladder have been defined, (including the critical role of calcium ion). The important new finding has been made that hyperplastic states in bladder epithelium can be controlled in vitro by addition of hydrocortisone to the culture medium in physiological doses. This response has been quantitated by both biochemical and morphological methods. The hyperplasia of bladder epithelium may be induced by treating animals with the selective bladder carcinogen, butyl (4-hydroxybutyl)nitrosamine. The bladder epithelium that is being cultured in vitro is thus known to have lesions which have potential to develop into true malignancies if left in the intact animal. The ability of retinoids to modify such hyperplastic states is currently being investigated; it is already well established from studies in other epithelia that there are significant synergistic effects between retinoids and adrenal steroids such as hydrocortisone.

Important studies on the ability of synthetic retinoids to prevent bladder cancer in the experimental animal have been completed during the past year. Transitional cell and squamous cell cancer of the bladder was induced in Wistar/Lewis female rats by direct instillation of N-methyl-N-nitrosourea into the bladder. Feeding of the synthetic retinoids, 13-cis-retinoic acid, inhibited the incidence and extent of bladder cancer in these rats, even when 13-cis-retinoic acid administration was begun after completion of the carcinogen treatment.

All of the above studies with retinoids suggest that this class of molecules has important activity in different types of epithelia, and that studies of mechanism of action in one target epithelium may provide valuable information about potential usefulness in other epithelia. With respect to the immediate goal of the Lung Cancer Branch, to prevent lung cancer in man, it is already clear that such studies of retinoid-dependent epithelia other than respiratory epithelium have provided important leads for new areas to be pursued in lung cancer research.

(4) Pharmacology and Biochemistry of Control of Epithelial Cell Differentiation with Retinoids - Since the possible eventual use of retinoids to prevent cancer in man is dependent on further understanding of the pharmacological properties of these agents, a new program has been established in the Branch to develop basic knowledge in this area, in which relatively little is known. New staff have been recruited during the past year and new methods are being developed. In particular, the new technique of high pressure liquid chromatography is being applied to investigate the metabolism and tissue distribution of retinoids. Initial efforts have been largely devoted to development of analytical methods. One area that has already received special attention is the problem of toxicity of retinoids, which limits the potential applications of some of these agents. It has already been found that the presence of a terminal carboxylic acid group in a retinoid molecule is often associated with highly toxic properties. Studies

pursued in the Branch during the past year have led to the conclusion that alteration of the acid function to a less polar derivative (such as an ether, an amide, or a hydrocarbon) may have the very desirable effect of lessening the toxic properties of the molecule, without appreciably diminishing the desired effects on cell differentiation. Moreover, it has been found that the pattern of tissue distribution of retinoids is markedly changed by alteration of the polar end group. This has the very practical result of "targeting" the retinoid to specific organs, such as lung, bladder, or breast, in which it is desired to prevent cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO-1-CP-04494-01-LC

PERIOD COVERED
September 27, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolism and Tissue Distribution Studies of Natural and Synthetic Retinoids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Anita B. Roberts Research Chemist LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to increase our understanding of the metabolism of retinoids. Such information must be had before a rational approach can be taken to the design of new retinoids with greater activity in the reversal of preneoplasia in epithelial tissues. The investigation is along two basic lines: 1) The study of the in vitro metabolism of retinoic acid and synthetic retinoids in subcellular systems of target organs, and 2) the study of the absorption, storage, and tissue distribution of synthetic analogs as compared to the natural retinoids.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: This is a new project. The purpose of the study is to investigate the metabolism of retinoids and from this to gain insight into the particular chemical modifications of synthetic retinoids which might enhance activity, lower toxicity, or change the tissue distribution of the compound. Part of this study is aimed at distinguishing between "activating" metabolic conversions and "deactivating" reactions which earmark a compound for elimination.

Methods Employed: An *in vitro* system for retinoid metabolism is being developed using subcellular fractions of target tissues such as tracheal, intestinal, bladder, or mammary epithelium. Ultracentrifugation is used to isolate subcellular fractions. Radioactive retiniods are used as substates.

The techniques employed for the detection of metabolites include freeze-drying, extraction, separation by high-pressure liquid chromatography (HPLC) by the solvent systems developed by Frolik et al. (submitted to J. Lipid Res.), and liquid scintillation counting.

Techniques for the study of the *in vivo* disposition of various retinoids involve an adaptation of a previously used extraction procedure to final analysis on HPLC. A new HPLC system has been developed for the analysis of samples with high lipid content.

Major Findings: The search for a system for the stydy of the metabolism of retinoids in subcellular fractions of target tissues is still in progress. Technical problems with tracheal epithelia have led to the choice of other epithelial tissues for initial experiments. The conditions necessary to minimize non-enzymatic breakdown of the retiniods and to maximize recoveries have been worked out.
In vivo experiments on vitamin A-deficient hamsters have shown that numerous synthetic retinoids are capable of being tranformed back into retinol, the parent compound, and therefore increase liver stores of the vitamin. It has been shown that this conversion to retinol and retinyl esters occurs in the intestine as well as in the liver.

Significance to Biomedical Research and the Program of the Institute: A greater understanding of the metabolism of retinoids should greatly influence the design of new retinoids with better therapeutic properties than the natural ones for treatment of prenoplasia. The development of the liver-storage assay has increased our ability to screen new retinoids and eliminate those that would increase liver storage and therefore not be useful for long-term clinical trials. It is hoped that this assay, coupled with the tracheal organ culture assay system developed by our Branch, can be used to find a non-polar retinoid that would be localized in fat, and could be used for the prevention of breast cancer in high-risk populations.

Proposed Course of Project

Future work on this project will involve: 1) further work on the development of a cell-free system for the study of retinoid metabolism in its target organs; 2) studies on the uptake and tissue distribution of several new radioactive retinoids currently being synthesized; 3) *in vitro* studies on the role of the liver in determining the fate of the retinoids in the animal.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04495-01-LC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Prevention of Experimental Bladder Cancer with Synthitic Retinoids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. Michael B. Sporn, Chief, LCB, NCI
Charles C. Brown, FSS, NCI
Robert A Squire, DCCP, NCI

COOPERATING UNITS (if any)

Martin Wenk, Microbiological Associates

LAB/BRANCH

Lung Cancer Branch, Carcinogenesis Program

SECTION

Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transitional cell and cell cancer of the bladder was induced in Wistar/Lewis female rats by direct instillation of N-methyl-N-nitrosourea into the bladder. Feeding of the synthetic retinoids, 13-cis-retinoic acid, inhibited the incidence and extent of bladder cancer in these rats, even when 13-cis-retinoic acid administration was begun after completion of the carcinogen treatment.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine the role of synthetic retinoids in prevention of experimental bladder cancer.

Methods Employed: The Hicks system, using intravesical administration of nitrosomethylurea (NMU) was used to induce bladder cancer in Wistar/Lewis female rats. Experimental animals were fed the synthetic retinoid, 13-cis-retinoic acid, to see if it would lessen the incidence of bladder cancer in rats treated with NMU.

Major Findings: There was a marked inhibition of bladder carcinogenesis by 13-cis-retinoic acid. There were significantly fewer neoplasms, as well as preneoplastic lesions, in the animals treated with this synthetic retinoid.

Significance to Biomedical Research and the Program of the Institute: These findings are of major importance, since they indicate that a synthetic retinoid can inhibit development of bladder cancer, even after dosing with carcinogen has been completed. The findings are of sufficient interest to justify consideration of clinical trial of this retinoid in high-risk human populations.

Proposed Course: To continue with further studies of 13-cis-retinoic acid and other synthetic retinoids for effectiveness in prevention of experimental bladder cancer.

Publications

Sporn, M.B., Squire, R.A., Brown, C.C., Smith, J.M., Wenk, M.L., and Springer, S. 13-cis-retinoic acid: Inhibition of bladder carcinogenesis in the rat. Science 195: 487-489, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04549-05-LC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Organ Culture Studies on Tracheobronchial Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael B. Sporn Chief, Lung Cancer Branch LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch, Carcinogenesis Program

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	0.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ability of vitamin A and its synthetic analogs (retinoids) to reverse a potentially premalignant lesion of tracheobronchial epithelium is being studied in organ culture. Keratinized, squamous metaplastic lesions are established in organ cultures of hamster tracheas. Addition of active retinoids causes reversal of these lesions and restoration of healthy mucociliary epithelium. Several new retinoids have been found to be extremely active and relatively free of toxicity, using this experimental system.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The principal objective is to study the direct effects of carcinogens and anti-carcinogenic agents (such as retinoids) on the cell population which is of immediate relevance for development of the most common form of human lung cancer, namely bronchogenic carcinoma. The principal experimental system that is being used measures the reversal of a potentially premalignant lesion, keratinized squamous metaplasia, by the addition of vitamin A and its synthetic analogs (retinoids).

Methods Employed: Classical methods of organ culture are being used. Tracheas are being obtained from either normal animals, from vitamin A-deficient animals, or from animals treated with respiratory carcinogens. After lesions have become established in organ culture, the cultures are treated with different retinoids. The restoration of a normal ciliated and mucus-producing epithelium is then measured.

Major Findings: The optimal conditions for long-term organ culture of tracheobronchial epithelium of vitamin A-deficient hamsters have previously been defined, using medium CMRL-1066.

New synthetic retinoids, such as retinylidene acetone, axerophthene, and retinoic acid 2-hydroxyethyl amide have been found to be highly active compounds for reversal of keratinized squamous metaplasia. Axerophthene has been found to be significantly less toxic than the natural retinoid, retinyl acetate.

Significance to Biomedical Research and the Program of the Institute: The finding that new analogs of retinyl acetate, with modifications in the side chain and terminal portion of the molecule, are directly active on the tracheal epithelium is particularly important. This finding opens the way for synthesis of new forms of vitamin A that may have greater pharmacological usefulness in man for prevention of lung cancer. Studies are currently in progress in the Lung Cancer Branch to evaluate the direct effects of synthetic retinoids on preneoplastic respiratory epithelium of hamsters.

Proposed Course of Project: To continue as outlined above.

Publications

Sporn, M.B. Approaches to prevention of epithelial cancer during the preneoplastic period. Cancer Res., 36: 2699-2702, 1976.

Sporn, M.B., Dunlop, N.M., Newton, D.L., and Smith, J.M. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (Retinoids) Fed. Proc. 35: 1332-1338, 1976.

Sporn, M.B., Dunlop, N.M., Newton, D.L., Henderson, W.R. Relationships between structure and activity of retinoids. Nature 263: 110-113, 1976.

Sporn, M.B. Retinoids and carcinogenesis. Nutrition Reviews 35: 65-69, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04573-03 LC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Control of Bladder Carcinogenesis with Retinoids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David H. Reese - Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch, Carcinogenesis Program

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the reversal of preneoplastic bladder lesions by retinoids (vitamin A and its analogs) in organ culture. Areas of specific interest include (1) the effects of retinoids on in vitro induced hyperplasia and dysplasia; (2) the effects of retinoids on in vivo carcinogen-induced hyperplasia and dysplasia; and (3) the functional relationships between retinoids and glucocorticoids.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04573-03-LC

Project Description

Objectives: The purpose of this project is to study the reversal of preneoplastic bladder lesions by retinoids (vitamin A and its analogs) in organ culture. Areas of specific interest include (1) the effects of retinoids on in vitro induced hyperplasia and dysplasia; (2) the effects of retinoids on in vivo carcinogen-induced hyperplasia and dysplasia; and (3) the functional relationships between retinoids and glucocorticoids.

Methods Employed: By culturing normal bladder in Ham's F-12 medium without serum, it is possible to induce hyperplastic and dysplastic epithelial lesions which resemble carcinomas in situ. Similar lesions can be induced in vivo by intragastric administration of the carcinogen butyl(4)-hydroxybutyl) nitrosamine. The ability of various agents to control these two types of lesions in vitro are then tested.

Major Findings: The hyperplastic response of bladder epithelium in F-12 medium has been quantitated. The number of cells in the bladder epithelium increases 3-fold during 7 days in culture. This hyperplastic response does not begin until after 1 day of culture. At 2 days of culture those cells which are in the process of DNA replication are predominately basal cells. At 3 and 4 days of culture, intermediate cells are seen replicating DNA. The hyperplastic response in F-12 medium can be blocked by hydrocortisone at physiological levels (10^{-8} M). Butyl (4-hydroxybutyl) nitrosamine-induced bladder lesions have been successfully cultured in Waymouth's MB752/1 medium. During a 4 day culture period, hyperplasia continues. This in vitro hyperplasia can be blocked by hydrocortisone.

Significance to Biomedical Research and the Program of the Institute: This is perhaps the only known system in which precancerous lesions, which have been induced in vivo, can be maintained and manipulated under defined organ-culture conditions. This system provides the opportunity to test agents (specifically retinoids) which may be capable of altering precancerous lesions. This system also has the capability of in vitro study, under defined conditions, of lesions closely resembling precancerous lesions. The development of these lesions can be inhibited. This is an excellent model system for development of methods and techniques for study of respiratory carcinogenesis in vitro.
Proposed Course of Project: The ability of various retinoids to either block development of in vitro induced lesions or to manipulate in vivo carcinogen-induced lesions will be studied.

Publications

Reese, D.H., Friedman, R.D., Smith, J.M., and Sporn, M.B. Organ culture of normal and carcinogen-treated rat bladder. Cancer Res., 36: 2525-2527, 1976.

Reese, D.H., Friedman, R.D., and Sporn, M.B.: Induction of hyperplasia and its suppression by hydrocortisone in organ-cultured rat urinary bladder. Cancer Res., in press.

Stinson, S.F., Lilga, J.C., Reese, D.H., Friedman, R.D., and Sporn, M.B.: Quantitation with an automated image analyzer of nuclear-cytoplasmic changes induced by hydrocortisone in bladder epithelium. Cancer Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04574-02-LC

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The effect of retinoids on carcinogenesis in the lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sherman F. Stinson Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Lung Cancer Branch, Carcinogenesis Program

SECTION

Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to define the effects of retinoids on carcinogenesis in the tracheal epithelium. To achieve this goal, several areas are being simultaneously investigated. 1) Different retinoids are being screened in long term in vivo studies to determine the maximum doses and effects of the retinoids. 2) A reproducible model for the study of lung cancer using N-methyl-N-nitrosourea (NMU), or other water soluble carcinogens, in the hamster trachea is being developed. 3) Methods for collecting exfoliated cells from the lung are being investigated.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The objective of this project is to accurately define an experimental model and establish methodology to study the effects of retinoids on preneoplasia in the lung.

Methods Employed: Syrian golden hamsters have been fed varying doses of 13-cis-retinoic acid for periods up to 4 months. Blood was collected at regular intervals for biochemical analysis, as were tissues for morphological study.

A cannula for the intratracheal instillation of soluble carcinogens as well as for the collection of exfoliated cells has been developed after a design of Dr. Hans Schreiber of Oak Ridge National Laboratory and the University of Chicago, and tests with this cannula in hamsters have been conducted. The carcinogen that will be used in these experiments is nitrosomethylurea (NMU).

Major Findings: Hamsters tolerate doses of 13-cis-retinoic acid up to 10 mg/week, but levels above this result in severe toxicity. Indications of toxicity include: weight loss, decreased food consumption, loss of hair, hyperkeratosis, and inflamation and decreased cornification of the esophageal epithelium.

The hamsters have been exposed to various levels of NMU, and carcinomas have been induced with 15 weekly treatments of a 1% solution of NMU. Cytology characteristic of cancer has also been collected and evaluated.

Significance to Biomedical Research and the Program of the Institute: These studies have significance in two major areas: The development of an animal model system for the study of lung cancer; the definition of the effects of retinoids on preneoplasia in the lung. The use of the new cannula system for intratracheal instillation of carcinogens is particularly important, since it allows direct application of carcinogen to a well-defined segment of the respiratory tract. This particular area then can be observed for the development of preneoplastic and neoplastic lesions, and factors that prevent the development of respiratory cancer can be intensively studied.

Proposed Course of Project: Other retinoids will be screened by in vivo toxicity studies. Detailed experiments on the dose-response of NMU carcinogenesis in the trachea using the fabricated cannula will be conducted. The effect of selected retinoids on NMU carcinogenesis in the trachea will be studied. The effect of retinoids on the initiation of neoplasia will be investigated in a DNA-carcinogen binding system. Other water soluble carcinogens, such as ethylnitrosourea, will be tested to try to find a more potent carcinogen with less toxicity due to acute tissue damage.

Stinson, S.F., Lilga, J.D., Reese, D.H., Friedman, R.D., and Sporn, M.B.: Quantitation with an automated image analyzer of nuclear-cytoplasmic changes induced by hydrocortisone in bladder epithelium. Cancer Res. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04584-02-LC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Control of Epithelial Cell Differentiation and Carcinogenesis by Vitamin A

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C.A. Frolik Senior Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch, Carcinogenesis Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ultimate goal of this project is to find an agent that will cause a reversal of preneoplasia in epithelial tissue. The compounds currently being investigated are vitamin A and its analogs (retinoids). In order to better understand the effect of retinoids on the differentiation of preneoplastic epithelial tissue three major areas are being studied. (1) The biochemical mechanism of action of vitamin A in the epithelial cell. (2) The metabolism of vitamin A with special emphasis on determining the final active form of the vitamin. (3) The search for analogs of vitamin A that display biological activity in the reversal of preneoplasia and, at the same time, show low toxicity in the animal.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: It is the purpose of this study to determine the role of vitamin A in the control of epithelial cell differentiation - both normal and carcinogenic. Special emphasis will be placed on the interaction of the natural retinoids with various vitamin A analogs in order to find a compound that displays high activity in the reversal of preneoplasia while also showing low levels of toxicity.

Methods Employed: In order to study the biochemical mechanism of action of vitamin A, an in vitro hamster trachea culture system is being used. This system allows an epithelial tissue which is responsive to vitamin A to be separated from the complicating effects of the whole animal. Initial experiments have concentrated on the protein synthesis that occurs during the early time period following vitamin A administration to a vitamin A deficient trachea in culture.

In order to study the metabolism of the various natural and synthetic retinoids, a method is needed that is capable of separating closely related labile compounds. Such a procedure utilizing reverse-phase high pressure liquid chromatography has been developed in our laboratory during the past year. Using this chromatgraphic system together with a lyophilization method for extraction of tissues and medium, the metabolism of retinoic acid is actively being investigated.

Major Findings: Initial experiments involved in the elucidation of the biochemical mechanism of action of vitamin A have concentrated in the area of protein synethesis. It has been shown that there is a specific protein (or proteins) which are labeled with radioactive amino acids in the absence of retinoic acid but not in its presence. This protein is found in the membrane, cellular debris fraction after subcellular fractionation. A change in the labeling pattern of this protein occurs as early as 12-16 hours after replenishment of retinoic acid to retinoid deficient trachea cultures.

Having developed during the past year a rapid method for separation of retinoids using high pressure liquid chromatography, the metabolism of retinoic acid has been under investigation. Evidence indicates that retinoic acid is converted in vitro to several more polar metabolites. The appearance of these metabolites is dependent on the presence of trachea tissue. Several of these in vitro formed compounds comigrate with in vivo synthesized compounds in two high pressure liquid chromatography systems suggesting that what is being observed in vitro is indeed what is also taking place in vivo.

Significance to Biomedical Research and the Program of the Institute: It is hoped that the results of this project will provide information that will lead to the development of an analog of vitamin A that displays biological activity in the reversal of preneoplasia in epithelial tissues and yet shows little or no toxicity in the whole animal.

Proposed Course of the Project: Future work on this project will make use of the methods developed to date to study (1) protein synthesis in the retinoid deficient and retinoic acid supplemented trachea - especially in regards to the protein(s) found in the retinoid deficient trachea that does not occur in the retinoic acid supplemented tissue. Special emphasis will be placed on the mechanism whereby retinoic acid controls the synthesis of this protein(s); (2) metabolism of the natural retinoid, retinoic acid, and of various synthetic analogs with special emphasis on determining the form of the retinoid which is responsible for the biological activity of the compound.

Publications

Frolik, C.F., Tavela, T.T., and Sporn, M.B. Separation of the natural retinoids by high pressure liquid chromatography. J. Lipid Res. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04585-02-LC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Application of automated image analysis to the study of preneoplastic changes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sherman F. Stinson Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch, Carcinogenesis Program

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The feasibility of using an objective automated image analysis system for the study of preneoplastic morphological changes is being examined. Areas under investigation include: 1) optimization of techniques for fixation, processing, and staining of tissues and cells for histological analysis; 2) quantitation of hyperplastic responses in a variety of epithelial types; 3) quantitation of nuclear-cytoplasmic ratios in histological and cytological preparations, and measurement of changes in these ratios in response to several chemical stimuli; and (4) detection of changes in nuclear size, shape and chromatin distribution, as well as cytoplasmic alterations, in cells exfoliated from tissues exposed to various chemical stimuli.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04585-02-LC

Project Description

Objectives: This study is designed to evaluate the potential of an automated image analyzer for quantitating minute changes in cellular and subcellular size, shape, number and density which may have application to the detection and study of preneoplasia.

Methods Employed: A variety of epithelial tissues (including urinary bladder, trachea, and breast), as well as exfoliated cells from the lung and cultured urinary bladders, have been processed for histological use and examined on a Bausch and Lomb "Omnicon" image analyzer. Several different fixatives and stains, and 2 embedding media have been evaluated for their ability to preserve and intensify various specific morphological parameters.

Major Findings: It has been determined that the Omnicon can accurately, precisely and efficiently estimate the degree of cellular hyperplasia in an epithelium by quantitating the number of nuclei, the cross-sectional area of nuclei, or the total epithelial cross-sectional area, depending on the properties of the tissue being examined. Measurement of nuclear-cytoplasmic ratios in the urinary bladder has also been successful, and visually undistinguishable changes in the ratios caused by chemically altering the cellular environment have been detected. Further, changes in nuclear and cytoplasmic sizes have been measured in cytology samples collected from tracheas exposed to carcinogens, which enable these samples to be distinguished from those that were unexposed.

Significance to Biomedical Research and the Program of the Institute: These studies will contribute to an objective, quantitated definition of preneoplasia. The studies may also contribute to early diagnosis of neoplastic changes.

Proposed Course of Project: The technology of sample preparation will continue to be refined. Further investigations into quantifiable parameters indicative of preneoplasia will be conducted. Studies on epithelial responses to chemical stimuli will also be expanded in an effort to detect early neoplastic changes.

Publications

Stinson, S.F., Sporn, M.B.: Use of an automated image analyser to quantitate cellular hyperplasia in urinary bladder epithelium. J. Microscopy, in press.

Stinson, S.F., Lilga, J.C., Reese, D.H., Friedman, R.D., and Sporn, M.B.: Quantitation with an automated image analyzer of nuclear-cytoplasmic changes induced by hydrocortisone in bladder epithelium. Cancer Res., in press.

SUMMARY REPORT

BIOASSAY OPERATIONAL UNIT

July 1, 1976 through September 30, 1977

The Bioassay Operational Unit is divided into three subunits: The Bioassay Program, the In Vitro Program, and the Information Program. Doctor Richard A. Griesemer is Acting Director of this Unit, which is formally part of the Collaborative Contracts Program. The Bioassay Operational Unit is dependent on personnel from the Carcinogen Bioassay and Program Resources Branch (Dr. Cipriano Cueto, Jr., Acting Chief), the Tumor Pathology Section of the Experimental Pathology Branch (Dr. Dawn Goodman, Acting Head), and the Office of the Coordinator for Environmental Cancer (Dr. Herman Kraybill, Scientific Coordinator).

BIOASSAY PROGRAM

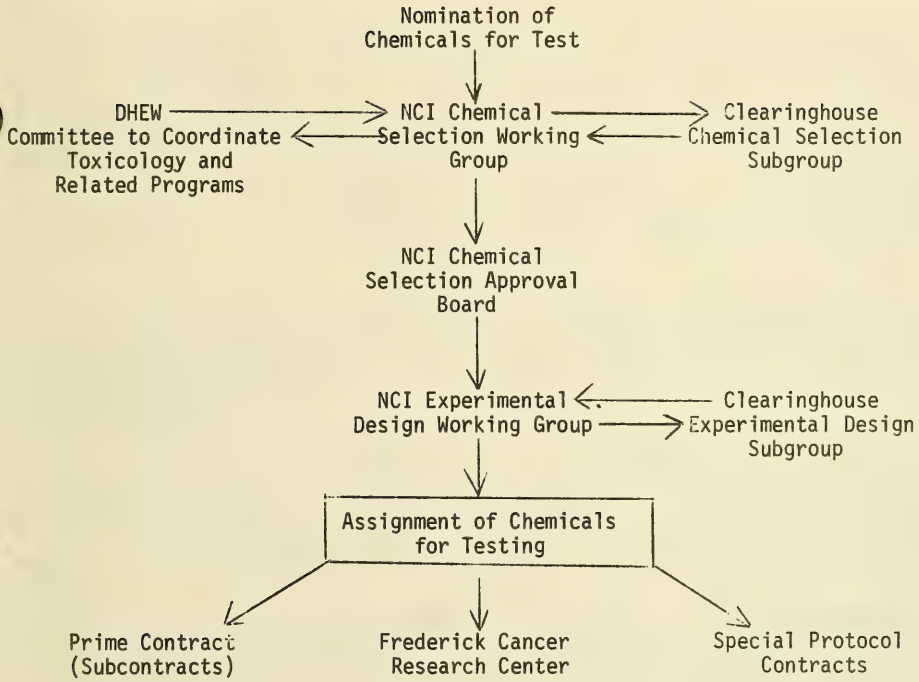
The Bioassay Program is responsible for implementing the decisions of the Working Groups of the National Cancer Institute (NCI) on Chemical Selection, Experimental Design, Pathology, and Data Evaluation. Overall coordination and administration of the Program's activities are provided by the Manager, Dr. Thomas W. Orme. The Bioassay Program acts both through a prime contract with Tracor Jitco, Inc. (NO1-CP-43350 - Dr. Marshall Steinberg, Principal Investigator and Dr. J. Fielding Douglas, NCI Project Officer) to whom defined tasks in the management and monitoring of carcinogenicity tests have been delegated and through contracts administered directly by NCI which are focused on testing methodology or on resource development. Emphasis is placed on the improvement of rodent tests, on the interpretation of the test results by teams of pathologists, on investigation of the biological nature of specific organ systems particularly susceptible to cancer, such as the mouse liver, and on writing test reports which provide part of the information required for assessment of the potential human risk posed by a tested chemical.

Plan of Operation

The Bioassay Program has two major goals: (a) to generate rodent bioassay data which can be utilized to identify the environmental chemicals potentially carcinogenic to man, and (b) to improve the design of rodent tests.

(1) New Chemicals to be Placed on Test - After the formation of the Clearinghouse on Environmental Carcinogens, an advisory committee chartered to render scientific comment on various aspects of this Program's activities, and the DHEW Committee to Coordinate Toxicology and Related Programs (CCTRP), chemicals tested under the auspices of this Program were divided into "old" and "new" categories. All "new" nominations of chemicals for test are subjected to a rigorous examination by the Clearinghouse, the CCTRP, and the NCI Working Groups on Chemical Selection and Experimental Design before tests begin. The procedure outlined in Figure 1 is an extension of former practice to include the newly chartered advisory groups and is designed to give greater public

Figure 1


Nomination of Chemicals for Test
DHEW Committee to Coordinate Toxicology and Related Programs
NCI Chemical Selection Working Group
Clearinghouse Chemical Selection Subgroup
NCI Chemical Selection Approval Board
NCI Experimental Design Working Group
Clearinghouse Experimental Design Subgroup
Assignment of Chemicals for Testing
Prime Contract (Subcontracts)
Frederick Cancer Research Center
Special Protocol Contracts

visibility to NCI supported carcinogen testing, in particular to the process of chemical selection. "New" chemical nominations selected by the NCI Chemical Selection Working Group for further consideration by advisory groups are listed in Table 1.

(2) Chemicals on Test - The list of chemicals on test is updated monthly and published by the information resources unit of the Carcinogen Bioassay and Program Resources Branch, under the coordination of Dr. Sidney Siegel. Tests have progressed to various stages ranging from experimental design, chemical procurement and chemical analysis to completion of histopathology, statistical analysis and report writing. In many cases tests have been completed, and reports are being written. For administrative purposes July 15, 1976 was selected arbitrarily to define the "backlog" chemicals. All chemical tests involving rodents sacrificed before July 15, 1976 and organized under the Prime Contract constitute the "backlog."

(3) Reports on "Backlog" Chemicals · A major effort is being made to publish reports on the "backlog" chemicals so that an assessment of potential risk to humans can be made by the Clearinghouse on Environmental Carcinogens and interested regulatory agencies. These reports are released by the Office of Cancer Communication as a Technical Report Series. Reports issued to date are listed in Table II.

To bolster the report writing staff of Tracor Jitco, Inc., the Mitre Corporation (NO1-CP-65863 - Dr. Lydia Thomas, Principal Investigator) was contracted to provide an independent supplementary technical writing team. The writing of reports is coordinated under a plan developed by NCI. Approximately 200 chemicals are covered by this plan.

(4) Mouse Liver Lesions - The Bioassay Program operates under the assumption that chemicals which induce hepatocellular carcinomas in B6C3F1 mice pose a potential carcinogenic threat to man. The economic impact of this assumption is great and the scientific argument for its validity is controversial. The Program is supporting research to characterize liver lesions which may be defined as neoplastic and which possess the ability to progress to carcinomas. In addition, data is being generated which may determine whether the etiology of these lesions involves mechanisms unique to mice. The research is being carried out under contracts with Universities of California (NO1-CP-65845 - Dr.Boris Ruebner, Principal Investigator), Maryland (NO1-CP-65752 - Dr. David Hinton, Principal Investigator) and Texas (NO1-CP-65846 - Dr. Frederick Becker, Principal Investigator).

(5) Central Support Facilities - Several contracts are funded which provide support to the testing program in rodent production (Harlan Industires, NO1-CP-65737 - Mr. Hal Harlan, Principal Investigator; and Charles River Breeding Laboratories, NO1-CP-55651 - Dr. George Pucak, Principal Investigator). These contracts are described in the narrative section.

(6) Other Research Activities - Two research-oreinted contracts are administered by the Bioassay Program because they involve extensive rodent testing. One (Temple University, NO1-CP-43271 - Dr. Donald Forbes, Principal Investigator) focuses on the filtration effects of ozone in

Table I

Chemicals Selected by the NCI Chemical Selection Working Group for Further Consideration by Advisory Groups*

Ammonium Alginate (low priority)
Antimony Trioxide
Benzene
Bromodichloromethane
Bromoform
N-Butylchloride
Castor Oil
Chlorendic Acid
Chlorowax 40
Chlorowax 500C
CN (Riot Control Gas)
Carboxylic Acid - Diethanolamine condensates of Coconut Oil Acids, Lauric Acid, and Oleic Acid
CS2 (Riot Control Gas)
Decabromobiphenyl Oxide
Dibromochloromethane
1,2-Dichloropropane
2,4-Dichlorophenol (low priority)
Diethanolamine
Gilsonite
Methylene Bromide
Monochloro-3,5-Xylenol
Monuron (low priority)
Nicotine
Nitrofurantoin
Oxalic Acid
Phenyl-Beta-Naphylamine
O-Phenylphenol
Propylbenzene (low priority
Pyridine
Rotenone
Sodium Fluoride
Succinic Acid
Tetrakis(Hyroxymethyl)Phosphonium Chloride and Sulfate
Witch Hazel
Xylene (Isomeric Mixture)

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*This list includes the chemical selections submitted to the Clearinghouse on Environmental Carcinogens for review as of April 28, 1977. A list of chemicals selected prior to formation of this Advisory Group is available from Dr. Sidney Siegel, Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

Table II*

"Backlog Reports" Published

Technical Report Series #1 - Guidelines for Carcinogen Bioassay in Small Rodents

Technical Report Series #2 - Carcinogenesis Bioassay of Trichloroethylene

Technical Report Series #3 - Bioassay of 1,1,1-Trichloroethane for Possible Carcinogenicity

Technical Report Series #4 - Bioassay of Dimethoate for Possible Carcinogenicity

Technical Report Series #5 - Bioassay of Proflavine for Possible Carcinogenicity

Technical Report Series #6 - Bioassays of Nitrilotriacetic Acid (NTA) and Nitrilotriacetic Acid, Trisodium Salt, Monohydrate ($Na_3NTA \cdot H_2O$) for Possible Carcinogenicity

Technical Report Series #7 - Bioassay of Phenformin for Possible Carcinogenicity

Technical Report Series #8 - Bioassay of Chlordane for Possible Carcinogenicity

Technical Report Series #9 - Bioassay of Heptachlor for Possible Carcinogenicity

Report on Carcinogenesis Bioassay of Chloroform

Report on Carcinogenesis Bioassay of Technical Grade Chlordecone (Kepone)

Reports on the following chemicals have been sent to the Office of Cancer Communications, National Cancer Institute for clearance and publishing:

Bioassay of Dichlorvos for Possible Carcinogenicity

Bioassay of EDTA for Possible Carcinogenicity

Table II* (Continued)

Aldrin/Dieldrin
Allyl Chloride
APC
Aroclor 1254
Azionphosmethyl
Captan
Chloramben
Chlorobenzilate
Chloropicrin
Chlorothanlinol
Dapsone
DBCP
Diarylanilide Yellow
1,1-Dichlorethane
Dichlorvos
Dieldrin
1,2-Dichloroethane
N,N-Dimethyl-P-nitrosoaniline
2,4-Dinitrotoluene
EDTA
Endosulfan
Endrin
Hexachlorophene
3,3'-Iminobis-1-propanol
Dimethanesulfonate (Ester)
Hydrochloride (IPD)
Isophosphamide
Lasiocarpin
Lindane
Malthion
2-Methyl-1-nitroanthraquinone
Mexacarbate
Nitrofen
Parathion
Phosphamidon
Pentachloronitrobenzene
Photodieldrin
Picloram
Procarbazine
1,1,2,2-Tetrachloroethane
Tetrachlorethylene
Tetrachlorvinphos
Toxaphene
1,1,2-Trichloroethane
Tris (2,3-Dibromopropyl) Phosphate

*As of June 15, 1977

blocking carcinogenic UV light and on the threat associated with atmospheric ozone depletion. The other (Howard University, NO1-CP-33266 - Dr. Govind Kapadia, Principal Investigator) is focused on the identification of carcinogens in herbs used commonly in man.

(7) Pathology Support - Several contracts developed by the Bioassay Program focus on the problems confronting pathologists who interpret bioassay results. The Experimental Pathology Laboratories (NO1-CP-65731 - Dr. William Busey, Principal Investigator) render professional advice to the NCI Pathology Working Group on a regular basis and have assumed the pathology workload in completing specific bioassays where this was not possible for the investigators to whom chemical tests had been assigned originally. A contract with the Johns Hopkins University (NO1-CP-65768 - Dr. John Strandberg, Principal Investigator) was also developed to support the activities of the NCI Pathology Working Group and the Tumor Pathology Section. An Institute for Laboratory Animal Resources program is supported by contract (NO1-CP-6580) and is directed toward resolution of nomenclature problems confronting veterinary pathologists.

(8) Frederick Cancer Research Center (FCRC) - This Program supports a multi-disciplinary project (FCRC - Project 8, Bioassay) in carcinogenesis research at FCRC directed by Dr. Richard Cardy. This project conducts bioassays by standard protocol and is developing methodology pertinent to the improved design of further bioassays.

IN VITRO PROGRAM

The major goals of the In Vitro Program are (1) to establish a battery or matrix of procedures which can be used as an effective pre-screen for establishing priorities for in-depth carcinogenicity testing, (2) to define the utility of in vitro test procedures for assisting in the evaluation of marginal data on carcinogenicity or mutagenicity derived from animal experiments, and (3) to provide research tools for investigation of the mechanism of action of chemical carcinogens, including identification of molecular targets and the obligatory steps of carcinogenesis.

In the past few years considerable research has been carried out to develop in vitro methods which can be used to determine the carcinogenic potential of chemical compounds. These in vitro assays can be broadly divided into three major categories, namely, those in which there is (1) induction of neoplastic transformation of mammalian cells in culture, (2) mutagenic or chromosomal changes in microorganisms or mammalian cells and (3) interactions between the chemical carcinogens and target macromolecules. Test systems from each of these categories are currently in the process of being defined and evaluated in terms of usefulness, reproducibility and comparability with known in vivo carcinogenicity.

Studies are under way to develop standardized protocols and to determine the predictive value of transformation systems using the (1) BALB/c 3T3 cell line (Arthur D. Little, Inc. - NO1-CP-55711), (2) primary and early passage Fischer rat embryo cells (Pfizer, Inc., John L. Smith Memorial for Cancer

Research - NO1-CP-55703), (3) Fischer rat embryo cells infected with Rauscher leukemia virus (Pfizer, Inc., John L. Smith Memorial for Cancer Research - NO1-CP-55703: and Microbiological Associates, Inc. - NO1-CP-55670), (4) hamster embryo cells (Frederick Cancer Research Center - NO1-CO-25423, (5) hamster embryo cells exposed to the compound transplacentally and then placed in culture (Stanford Research Institute - NO1-CP-55701), and (6) liver and skin epithelial cells (American Health Foundation - NO1-CP-55705; Children's Hospital of Akron - NO1-CP-55706; and University of North Carolina - NO1-CP-55707).

Each system poses its own experimental difficulties but the following aspects are being considered in the stepwise evaluation of each assay: (1) definition of the culture conditions which will yield the highest rate of transformation; (2) determination of the reproducibility and predictive value of the assay; (3) determination of the ability of the cells to activate procarcinogens to their ultimate reactive form; (4) evaluation of endpoints, other than morphology which would be valid criteria for in vitro transformation; and (5) incorporation of exogenous activation systems for those procarcinogens requiring activation through metabolic pathways not operational within the cells in culture.

Although the correlation between mutagenicity and carcinogenicity has more than adequately been demonstrated with the Salmonella typhimurium strains developed by Dr. Bruce Ames, a broad-based assessment of the predictive value of microbial systems through coordinated studies in several laboratories was considered necessary and is under way. The laboratories participating in this study are Inveresk Research International (NO1-CP-65858), Litton Bionetics, Inc. (NO1-CP-65856), New York Medical College (NO1-CP-65855), and Stanford Research Institute (NO1-CP-65857). Approximately 80 compounds including carcinogens, non-carcinogenic analogues and compounds tested in the NCI Bioassay Program will be evaluated, both with and without metabolic activation, in mutagenicity assays using five Salmonella typhimurium strains (TA-1535, 1537, 1538, 98, and 100) and one Escherichia coli strain (WP2uvrA$^-$), and (2) DNA repair assay using Escherichia coli strains W3110/pol A$^+$ and p3478/pol A$^-$. The metabolic activation systems used in this study are being derived from the livers of both uninduced and arochlor 1254 induced Fischer 344 rats, B6C3F1 mice (C57B16XC3H[MTV-F_1]) and Syrian hamsters.

In addition to the microbial mutagenicity assays, developmental and evaluation studies are proceeding with a mammalian mutagenesis assay using L5178Y/TK$^{+/-}$ mouse lymphoma cells. These studies are being carried out by Litton Bionetics, Inc. (NO1-CP-65853) and Stanford Research Institute (NO1-CP-65854). The developmental phase includes definition of test conditions and determination of the utility of rodent liver metabolic activation. In the evaluation phase, blind studies with the same chemical compounds used in the microbial assays will be carried out.

Although initial emphasis within the In Vitro Program is on development and validation of various short-term assays, there are a series of contracts which support research on the development of in vitro carcinogenesis models and mechanisms of action. These studies are as follows: (1) determination

of the process by which chemical carcinogens promote viral oncogenesis and whether oncogenic and non-oncogenic viruses may promote transformation by known chemical carcinogens in the same way (Biolabs, Inc. - NO1-CP-45615); (2) comparison of the metabolism of carcinogens by human and rodent tissues and reactivity of carcinogens and their metabolites in mutagenesis and cell transformation assays (International Agency for Research on Cancer - NO1-CP-55630); (3) development of both an initiation/promotion system and an assay of syn-carcinogenesis for combinations of weak carcinogens in the C_3lt 10T1/2 cell line (University of Southern California - NO1-CP-65831); (4) determination of the characteristics and responsiveness of the BHK21 hamster cell system (University of Illinois - NO1-CP-23303); and (5) evaluation of human cell culture systems for in vitro carcinogenesis studies and the role of hormones as inhibitors or promotors and viruses as promoters of carcinogenesis (Ohio State University - NO1-CP-43276).

As support for those contractors using hamsters and hamster embryo cells, the Leo Goodwin Institute for Cancer Research (NO1-CP-65804) maintains a breeding colony and supplies Syrian hamsters.

INFORMATION PROGRAM

The Information Program provides services and support to the bioassay activities of the Carcinogenesis Program. These support functions have ranged from production of various publications to setting standards and specifications for machine-readable files and data systems and assuming responsibility for the design of special computerized information systems.

Several publications are supported under the contracts. Carcinogenesis Abstracts is a secondary publication which is produced by the Franklin Institute Research Laboratories (NO1-CP-43293). During the past year, the publication has been prepared at an accelerated rate and all of the 1976 volume will be produced by June 1977. A formal survey of users of the Journal was conducted by the MITRE Corporation. There was a 32% response rate for researchers, administrators, physicians, teachers, library personnel, and students. Results showed that the Journal is frequently and thoroughly used, that the format and coverage of the Journal are satisfactory, and that microfilm or computer data bases only would markedly diminish its effectiveness. (Abstracts from the Journal are already entered into the National Library of Medicine's CANCERLINE.) Users would be willing to pay a yearly subscription price, if need be, in order to have their own copy. This year a request for proposal was written for the production of Carcinogenesis Abstracts by the contractor who will then advertise and sell the Journal on a subscription basis.

Other publications which have an impact on both public health and research activities are the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man (International Agency for Research on Cancer - NO1-CP-45608). The production of these publications is partially funded by the Information Program. During the year, Volume 13 (Some Miscellaneous Pharmaceutical Substances) and Volume 14 (Asbestos) were published. Volume 15 (Some Fumigants, Miscellaneous Industrial Chemicals and Herbicides 2, 4-D

and 2, 4, 5-T and chlorinated dibenzodioxins) is in press. Each monograph is prepared by an international group of experts and serves as an authoritative source on the compounds which they contain. The IARC Information Bulletin on the Survey of Chemcials Being Tested for Carcinogenicity is also supported from this contract. Issue 6 of this series has been published and distributed to subscribers.

Stanford Research Institute (NO1-CP-33285) is completing the fourth year of a five-year incrementally funded contract. The objective of this project is to determine those chemicals with which man comes in contact, place them in "exposure categories" and make data and information available on their structure, production, distribution, and exposure level. Exposure categories presently under development are agricultural chemicals, adhesives and sealants, and chemicals with occupational exposure hazards.

The Environmental Mutagen Information Center (EMIC) (YO1-CP-2303) is partially supported by funds from NCI. EMIC is part of the Energy Research and Development Administration (ERDA), Oak Ridge National Laboratory. The mission of EMIC is to collect, organize, and make available chemical mutagenesis information gleaned from world-wide scientific literature. As more knowledge has accumulated about the structure and metabolic fate of chemicals with respect to their biological activity, a general qualitative correlation between mutagenicity and carcinogenicity has been made. The idea of using certain chemical mutagenicity assay systems as prescreens for chemical carcinogens has been endorsed by the Subcommittee on Environmental Carcinogenesis of the National Cancer Advisory Board.

A contract with EG/G Mason Research Institute (NO1-CP-43257) provides support services for the Carcinogenesis Bioassay Data System. The contractor maintains automated files for the contract, chemical, and bioassay information which are used extensively to help scientists, legislators, consumer advocates, legal organizations, manufacturers, and other interested groups.

The Franklin Institute (NO1-CP-65810) Systems Sciences, Inc. (NO1-CP-65760), Tracor Jitco, Inc. (NO1-CP-65809), Enviro, Inc. (NO1-CP-65808) and Illinois Institute of Technology Research Institute (NO1-CP-65811) were available during the year to supply a means for handling information needs requiring quick or custom-tailored response. Four tasks were continued from last year and four new ones were initiated this year.

new project, Computer Aided Prediction of Metabolites for Carcinogenicity Studies was started with the University of California at Santa Cruz (NO1-CP-65816). Based on the premise that otherwise harmless compounds can be converted through body metabolism into toxic and possible carcinogenic agents, the contractor has devised a computer program to help predict what these metabolites might be. A data-gathering stage is under way and a committee of NCI experts has been formed to gather information on metabolic reactions.

An interagency agreement has been established between the National Center for Toxicological Research (Food and Drug Administration and Environmental Protection Agency) at Pine Bluff, Arkansas, and the National Cancer Institute

(YO1-CP-70201) to analyze, design, and implement a computer-based system that will be used to capture, analyze, and report data and information relevant to the Carcinogenesis Bioassay of Chemicals. Toward the end of this fiscal year, the analysis phase was initiated. Each of the next two phases will be treated under separate contracts.

Because of the short duration of each of the last two contracts, no narratives appear.

CONTRACT NARRATIVES

BIOASSAY OPERATIONAL UNIT
Bioassay Program

July 1, 1976 through September 30, 1977

CALIFORNIA, UNIVERSITY OF (at Davis) (NO1-CP-65845)

Title: Biology of Neoplastic Liver Lesions in Mice

Contractor's Project Director: Dr. Boris Ruebner

Project Officer (NCI): Dr. Richard A. Griesemer

Objectives: To investigate the neoplastic potential of the nodules produced in mice by some compounds in the environment which may be carcinogenic. The morphologic, biochemical and biologic characteristics of the entire range of hepatic lesions produced by such a compound (Dieldrin) will be studied and compared with the lesions produced by a powerful carcinogen, diethylnitrosamine. Transplantation into nude mice as well as metastasis will be used as principal methods to study the biology of the lesions. It is also hoped to identify diagnostically useful morphologic and biochemical markers for premalignant lesions and also to identify potentially reversible lesions.

Significance to Biomedical Research and the Program of the Institute: The mouse is employed commonly in carcinogen bioassay. There is an important group of chemical compounds which produce hepatic lesions in mice but scarcely, if at all, in other animals. The neoplastic potential of these lesions has been disputed. It is hoped that this project will contribute towards a better understanding of the significance of these lesions.

Proposed Course: Male C3H mice, a strain with a high spontaneous hepatoma incidence and male C57BL/6, a strain with a low incidence are being used. Groups of 200 animals of each strain were treated with 12 mg/kg of diethylnitrosamine, a strong carcinogen. This was given in January 1977. Additional groups of 200 animals of each strain have been fed 10 ppm of Dieldrin, a weak carcinogen, since January 1977. The controls consist of 200 mice of each strain which have not received any carcinogen.

At 1 and 2 months, six animals of each group have been bled by orbital sinus puncture and sacrificed. At 3 months, three animals were bled and sacrificed. Complete autopsies were performed. Livers have been studied grossly, histologically, and by histochemistry. So far no early potentially malignant lesions have been detected.

Date Contract Initiated: September 30, 1976

Current Annual Level: $158,404

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CP-55651)

Title: Establishment of a Gnotobiote Originated Rodent Production Colony

Contractor's Project Directors: Dr. Albert P. Otis
Dr. George J. Pucak

Project Officer (NCI): Dr. Thomas Cameron

Objectives: Independently and not as an agent of the Government, Charles River shall establish breeding colonies to produce Fischer inbred 344 rats at a level of 2,000 per month and B6C3F1 hybrid mice at the projected level of 4,000 per month. Breeders for the "barrier" breeding colonies will be supplied by pedigreed expansion colonies of association flora status animals which will be maintained in isolator systems. The major objective of this program is to supply the Government with animals of defined quality in sufficient numbers to maintain the level of the Carcinogenesis Program.

Major Findings: Because of the exactness required to develop sufficient pedigreed isolator stock for this program in addition to those germfree animals supplied by the Government, the Contractor had to caesarian derive in germfree isolators additional pedigreed animals to increase its capability to meet the increased requirements by the Government. In addition to the initial breeding stock necessary to support this effort, a recycling program will take place on a routine basis in order that there not be a genetic shift from the animals produced by the Contractor and those maintained at NCI. The current goal to support the production requirements noted above would require associated pedigreed isolator foundation stock bred on a one to one basis of 60 pair of F344 inbred rats, 240 pair of C3H/He and 420 pair of C57BL/6. When the quantities required in the foundation colonies are reached, 500 pair of F344s should be sufficient to produce the 2,000 rats required monthly. The C57BL/6 females x the C3H/He males at a paired level of 1,200 should produce 4,000 B6C3F1 hybrid monthly.

Significance to Biomedical Research and the Program of the Institute: It is felt that producing animals from isolator derived colonies should enable researchers to utilize animals of uniform quality with little or no variability. The major reason for enabling this to be accomplished is the maintenance of the breeding stock for the production colonies in germfree isolator systems. It will also provide assurance that should difficulty arise in the production colonies, defined animals can be made immediately available for production replacement.

Proposed Course: The program as outlined in the contract is progressing within the limitations of the associated germfree pedigreed foundation stock. It appears that the production goal of this contract, which was significantly altered from its origianl date of October 1, 1974, by modification of June 29, 1976, will be reached by November 1, 1977.

Date Contract Initiated: October 1, 1974

Current Annual Level: $219,408

CLEVELAND METROPOLITAN GENERAL HOSPITAL (NO1-CP-65840)

Title: Support of a Mouse Colony with Liver Tumor Incidence

Contractor's Project Director: Dr. Brian Helyer

Project Officer (NCI): Dr. Dawn Goodman

Objectives: This contract was developed to provide maintenance support for the dispersal of a colony of CBA/H-T6T6 mice characterized by a high spontaneous incidence of hepatocellular carcinoma.

Date Contract Initiated: August 1, 1976

Current Annual Level: $3,000

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. (NO1-CP-65731)

Title: Animal Pathology Support

Contractor's Project Director: Dr. William M. Busey

Project Officer (NCI): Dr. Dawn Goodman

Objectives: (1) To provide consultation services to the Chief of the Tumor Pathology Section; (2) to perform the histopathological evaluation of animal tissues from intramural and extramural programs within the Carcinogenesis Program; and (3) to provide training in histologic techniques for contractor personnel in the Carcinogenesis Program.

Major Findings: During the past year, a histopathologic evaluation of rats and mice receiving piperonyl butoxide, piperonyl sulfoxide, ethyl tellurac, and 2,6-di-t-butyl-4-methylphenol (BHT) was conducted. Histopathologic evaluation of mice receiving amanthrene was also conducted. Preliminary evaluation of these studies indicate that piperonyl butoxide and BHT have no carcinogenic effect in rats or mice. Piperonyl sulfoxide appears to induce hepatolcellular carcinomas in mice, but is not carcinogenic to rats. The results of the ethyl tellurac studies in rats and mice are currently being reviewed at NCI.

The pathologists at Experimental Pathology Laboratories participated in the conduction of two NCI-Tracor Jitco workshops during the past year. At both workshops, the presentations concerned the preparation of pathology narratives.

The pathologists at Experimental Pathology Laboratories made five site visits during the past year. The first was to the National Center for Toxicologic Research. The purpose of the visit was to observe NCTR's automated pathology

reporting system. Other site visits were made to Battelle Columbus Laboratories, IITRI, Industrial Bio-Test Laboratories, and Mason Research Institute. During these site visits, Experimental Pathology Laboratories' pathologists discussed in detail the format and content of the pathology narrative required by NCI.

The pathologists at Experimental Pathology Laboratories participated in the activities of the Pathology Working Group. During the past year, the pathologists reviewed slides and pathology narratives submitted to NCI. Experimental Pathology Laboratories' pathologists also assisted in the preparation of a modified version of the Systematized Nomenclature of Pathology. This document, referred to as the "Mini-SNOP", has recently been alphabetized and is currently being used in the Carcinogenesis Program.

Significance to Biomedical Research and the Program of the Institute: This Animal Pathology Support contract provides a great deal of flexibility to the Carcinogenesis Program contractors and to the Chief of the Tumor Pathology Section. The personnel and facilities of Experimental Pathology Laboratories are available to the Program contractors and to various intramural investigators in the Program. The pathology services provided by Experimental Pathology Laboratories allow for the timely completion of the histopathologic evaluation of sensitive compounds under investigation in the Carcinogenesis Bioassay Program. Intramural investigators within the Carcinogenesis Program may use the facilities and personnel of Experimental Pathology Laboratories to assist them in the conduction of their research programs. The pathologists at Experimental Pathology Laboratories are available for consultation, and the histology laboratory of Experimental Pathology Laboratories is available for the processing of animal tissues for histologic examination. Similarly, the histology laboratory at Experimental Pathology Laboratories is available for the training of intramural and extramural histology technicians.

Proposed Course: To continue this pathology support service to the Carcinogenesis Program for as long as it is required.

Date Contract Initiated: April 20, 1976

Current Annual Level: $484,000

HARLAN INDUSTRIES, INC. (NO1-CP-65737)

Title: Establishment of a Rodent Production Colony

Contractor's Project Director: Mr. Hal P. Harlan

Project Officer (NCI): Mr. Clarence Reeder

Objectives: To develop a rodent production colony housed under strict barrier conditions in the shortest possible time, and to attain an issuable weanling level of 2,000 Fischer Inbred 344 rats and 4,000 B6C3F1 hybrid

mice of equal sex on a monthly basis. Pedigreed colonies are housed in associated isolators and are self-sustaining, germfree pedigreed litters will be furnised to the contract on a yearly basis from the genetic unit of NIH.

Major Findings: Associated pedigreed colonies of inbred Fischer 344 rats and C3H inbred mice have been established at the desired level. The C57BL6 inbred mice from the associated isolator colonies are progressing nicely, and production is slightly ahead of schedule. The Fischer 344 rats are now being introduced into our barrier continually, and to date no major problems have been detected.

Significance to Biomedical Research and Program of the Institute: The significance of this contract is that it will provide a constant and desirable source of animals to the various NCI contractors, enabling them to continue their programs which utilize the strains being produced. It will also insure the use of a standard biological mode to those investigators performing related experiments.

Date Contract Initiated: June 24, 1976

Current Annual Level: $447,410

HOWARD UNIVERSITY (NO1-CP-33266)

Title: Chemical and Biological Investigation of Potential Carcinogens from Plants

Contractor's Project Directors: Dr. Govind J. Kapadia
Dr. S. N. Pradhan

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: (1) To prepare purified fractions from certain herbs that are used habitually in Curacao and South Carolina and isolate and characterize crystalline compounds from these fractions, (2) to test each fraction and compound for carcinogenicity in experimental animals and identify substances responsible for the carcinogenic effects, (3) to extend such chemical and bioassay studies to plant species related to the above as well as to other plants that are suspected of possessing carcinogenic activity, and (4) to elucidate the chemical structure of any carcinogenic substance that may be isolated.

Major Findings: This project is a continuation of an earlier investigation which was developed in an attempt to correlate the high incidence of esophageal carcinoma in natives of certain places with their habit of using herbaceous folk remedies. Thus far 61 plant samples (belonging to 57 genera and 60 species, procured from Curacao, South Carolina and other places) have been extracted with water, the aqueous extracts lyophylized and submitted for

bioassay. The aqueous extracts of tannin-bearing plants were fractionated to yield tannin and tannin-free fractions which were also provided for bioassay.

Four bioassay subcutaneous injections in suitable doses were given to 15 male and 15 female NIH black rats once a week up to 72 weeks. The observation period ran up to 90 weeks. Earlier results of bioassay on a total of 24 extracts and fractions from twelve plants were reported and several of the products tested were found to be tumorigenic. Among these plants, Acacia villosa appeared to be the most potent carcinogen. The most significant part of these results was that the rats developed tumors when tea (Camellia sinensis), which is commonly consumed as a beverage, was administered subcutaneously. The induced tumors were malignant fibrous histiocytomas similar, if not identical, to those encountered in humans.

In the subsequent studies total aqueous extracts of twelve plants were used. The results of bioassay were: Paederia foetida (0/30), Baccharis halimifolia (0/30), Psidium guava (2/30), Areca catechu (30/30), Rhus copallina (10/30), Gnaphalium obtusifolium (0/30), Saccharum sp., blue ribbon variety (0/30), Chenopodium ambrosiodes (16/30), Diospyros virginiana leaves (17/30), Hamamalis virginiana (3/30), Sassafras albidum, safrole free extract (20/30), Myrica cerifera, leaves (0/30). Among these plants, Areca catechu total aqueous extract produced tumors at the injection site in 100% of the treated animals.

Chemical investigations of the plant extracts which produced tumors were undertaken. Results of the studies on Sassafras albidum, Myrica cerifera and Melochia tomentosa roots which afforded several compounds, were reported earlier. In continuation studies, characterization of earlier isolated unidentified constituents was performed. Among the compounds characterized included, 6,methoxy-7,8-methylenedioxycoumarin, two new cyclopeptide alkaloids, melonovines A and B and the known scutianine B, as well as an unusual quinolinone alkaloid melochinone. Besides these, a new quinolinone, designated melovinone, which is characterized as an open chain analog of melochinone, has been isolated. Furthermore, isolation and characterization of phenylpentylisatins - a novel class of alkaloids - has been realized. Among these include two isatin products designated as melosatins A and B, the former being characterized as 6,7-dimethoxy-4-(5'-phenylpentyl) isatin and the latter as 4-(5'-phenylpentyl) isatin. The structure of melosatin A has been corroborated by its total synthesis. The compounds isolated in these studies are being investigated for their carcinogenic potential. Extracts of four plants, including Camellia sinensis, which produced tumors in rats in subcutaneous studies, are prepared to provide for oral feeding studies in rats.

Significance to Biomedical Research and the Program of the Institute: This project is directly related to the major objective of the Carcinogenesis Program, i.e., the identification of unknown carcinogens in natural plant materials. This project should serve as the ultimate check to ascertain the carcinogenicity of materials implicated by epidemiological studies on the island of Curacao and in South Carolina. Information gained from this study could conceivably lead to definitive studies that should be conducted in other high incidence areas to identify the carcinogens involved.

Proposed Course: It is proposed to isolate and characterize chemical compounds in plant extracts found to be carcinogenic. Also, several of those plant extracts which have a higher incidence of tumors in animals by subcutaneous injection, will be administered orally with a view to simulating the natural route of consumption of these products.

Date Contract Initiated: June 25, 1971

Current Annual Level: $142,510

JOHNS HOPKINS UNIVERSITY (NO1-CP-65768)

Title: Animal Pathology Support

Contractor's Project Directors: Dr. John D. Strandberg
Dr. Linda C. Cork

Project Officer(NCI): Dr. Dawn G. Goodman

Objectives: This project provides pathology support to the intramural and extramural pathology programs using histochemistry, light and electron microscopy. The contract pathologists participate in advisory groups and workshops for the Bioassay Program and for other segments of the Carcinogenesis Program. They study experimentally produced lesions with a view to ascertaining the progression of the lesions and identification of preneoplastic states. Lesions will also be studied from a comparative viewpoint to document morphologic and functional similarities to spontaneous tumors of man and animals. Finally, this program supports training for veterinarians and physicians in the field of tumor and comparative pathology.

Major Findings: The pathologists regularly particpate in an informal working group which critically evaluates the pathology in the Bioassay Program. Several particular neoplasms are under morphologic and histochemical study. These include murine hepatocellular carcinomas and a series of spindle cell neoplasms which have arisen in both rats and mice. Several major groups have been identified including malignant fibrous histiocytomas and lesions compatible with foreign body sarcomas. The pathologists have contributed to the development of a dictionary for use with the computerized SNOP system used to code pathology data. This dictionary is for use by the Bioassay Program and should greatly help standardize the pathology terminology and decrease inconsistencies in diagnoses.

Significance to Biomedical Research and the Program of the Institute: The activities reported here help standardize and upgrade the level of pathology in the Carcinogenesis Program. This is especially important at a time when carcinogenesis testing is under close public scrutiny. The investigative aspects contribute to knowledge in the field of experimental carcinogenesis. Training pathologists will increase the number of qualified individuals in this field which has a severe manpower shortage.

Proposed Course: Service, investigative, and training aspects will continue. Specific projects include surveillance of a colony of Mastomys species for spontaneous neoplasms, completion of a study of hycanthone induced liver lesions in mice, and further comparative studies of spindle cell tumors in rodents. Pathology training will also continue and a new fellow will begin training on June 1, 1977.

Date Contract Initiated: March 22, 1976

Current Annual Level: $145,650

MARYLAND, UNIVERSITY OF (NO1-CP-65752)

Title: The Biology of Neoplastic Liver Lesions in Mice

Contractor's Project Directors: Dr. David E. Hinton
Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Richard A. Griesemer

Objectives: This project seeks to determine the biological characteristics of liver tumors induced in mice. To achieve this goal, in vitro cell and organ culture, enzyme histochemistry, tumor transplantation, intermediate energy metabolite, and morphologic assays will be used. Biological characteristics of tumors in the livers of BALB/C mice produced by compounds (i.e., Dieldrin, DDT) which have a negative or marginal effect at other organ sites and in other species will be compared to tumors produced by a carcinogen (Safrole) which is known to cause unequivocal liver cancer in the mouse and in the rat. Early liver lesions will be defined and their relationship to development of overt hepatocellular carcinoma will be achieved.

Major Findings: Qualitatively similar responses have been demonstrated in livers of BALB/C mice fed DDT, dieldrin, or safrole. When compared to controls, livers from animals fed the experimental diets for 14 days showed elevations in liver weight and liver/body weight ratios. Cytochrome P-450 and NADPH-cytochrome c reductase were increased. After 30 days, aniline hydroxylase and aminopyrine demethylase were increased as well. Liver lipid was increased in all exposure groups; however, animals fed safrole had most severe changes.

Significance to Biomedical Research and the Program of the Institute: Since the mouse is one of the most commonly used animals in carcinogenesis research, including long term bioassay, an understanding of the biology of neoplastic liver lesions in this species is important. Comparison of biologic properties of neoplastic liver lesions should lead to the determination of whether unifying criteria for classification exist.

Proposed Course: Long term dietary exposure of male BALB/C mice to DDT, Dieldrin, and safrole will be done to produce tumors for biologic study and comparison. Serial exposures will be carried out to determine stages in neoplastic development. Finally, studies will be done to determine which of the lesions produced can be reversed by cessation of exposure.

Date Contract Initiated: September 30, 1976

Current Annual Level: $63,564

MITRE CORPORATION/METREK DIVISION (NO1-CP-65863)

Title: Preparation of Carcinogenesis Bioassay Reports

Contractor's Project Directors: Dr. Lydia Thomas
Dr. Mary Kornreich

Project Officer (NCI): Dr. Thomas P. Cameron

Objectives: The data generated from the compounds tested during the backlog carcinogenesis bioassays are to be compiled, collated, and presented first for review and subsequently as camera-ready copies, to the Data Evaluation Group (DEG) of the Carcinogen Bioassay and Program Resources Branch of NCI. This contract, therefore, requires that METREK sort through environmental data records, individual laboratory project records, daily laboratory records, etc., to establish and record the design of the experiment. Then the clinical, pathologic and statistical evaluations as supplied, respectively, by the performing laboratory personnel, examining pathologist(s), and Tracor Jitco statistician are incorporated. The history of animal survival is supplied with the statistical evaluation and from this data and from laboratory animal weight and food consumption records, graphic representations of these are prepared by METREK for inclusion in the document.

Major Findings: The contract effort described above has been integrated into NCI's masterplan for report writing and is under constant review by the NCI Data Evaluation Working Group.

Significance to Biomedical Research and the Program of the Institute: The availability of these reports will directly impact the ability of regulatory agencies, manufacturers, and the public to draw conclusions pertaining to the continued use or need for further testing of the compounds assayed. Therefore, it is important that the results be accurately recorded in a timely manner. The METREK Division of The MITRE Corporation is assisting in accomplishing this goal.

Proposed Course: A continuation of the report preparation process as orignially conceived is contemplated at this time.

Date Contract Initiated: September 30, 1976

Current Annual Level: $355,689

NATIONAL ACADEMY OF SCIENCES/INSTITUTE OF LABORATORY ANIMAL RESOURCES
(NO1-CP-65805)

Title: Histologic Classification of Laboratory Animal Tumors

Contractor's Project Director: Dr. Earl W. Grogan

Project Officer (NCI): Dr. Dawn Goodman

Objectives: To formulate a tentative histological classification and nomenclature for laboratory animal tumors.

Major Findings: The ILAR assembled a committee of experts to review current programs and publications dealing with histologic classification and nomenclature of laboratory animal tumors. It was the committee's findings that the present programs do not fully meet the needs for standardized terminology and histologic classification of laboratory animal tumors. After a discussion of priority of needs it was decided that rat liver tumors should be the first subject of an effort, to develop a tentative histologic classification and nomenclature. The committee further concluded that it would be in the best interests of the study if a subcommittee consisting of pathologists intimately involved in the pathology of rat liver tumors be appointed to develop the report. The subcommittee was appointed in December 1976.

Significance to Biomedical Research and the Program of the Institute: There is within the biomedical community a lack of uniformity in identifying and classifying pathological lesions, particularly laboratory animal tumors as they relate to the tumors in man. The frequency and nature of tumors in the body sites differ markedly among the various species, breeds, and regions. The histologic terminology applied to these pathological lesions is used loosely; in many published reports, it is not clear whether investigators in different laboratories are observing the same tumors, or whether uniform nomenclature is being used to describe similar tumors in different species. Articles on the histology and classification of tumors tend to be widely scattered in the scientific publications; many are not easily obtainable. This circumstance, in the face of a continuing rise in the number of compounds being tested for carcinogenicity (wherein carcinogenicity is determined on the basis of tumors found in experimental animals) makes it imperative that the histologic classification and nomenclature of laboratory animal tumors be standardized.

Proposed Course: The subcommittee is developing an atlas on rat liver tumors which will include both benign and malignant tumors and associated lesions as well as pertinent references. The material covered in the atlas will be illustrated by predominantly black and white photographs with color illustrations of selected features, such as histochemistry. Current planning calls for final publication of the report as a monograph.

Date Contract Initiated: June 30, 1976

Current Annual Level: $40,000

NCI-FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CP-25423)

Project #8

Title: Bioassay Research

Contractor's Project Director: Dr. Richard Cardy

Project Officer (NCI): Dr. Richard Griesemer

Contract narrative is reported under the Interdisciplinary Programs and Resources Operational Unit.

TEMPLE UNIVERSITY (NO1-CP-43271)

Title: A Study of UV Carcinogenesis

Contractor's Project Director: Dr. Paul D. Forbes

Project Officer (NCI): Dr. Henry Hennings

Objectives: The primary objective of the investigation is to identify and quantitatively assess the relationship between changes in the "effective thickness" of stratospheric ozone and the development of solar damage to the skin, particularly carcinogenesis. The basic experiment is designed to give data based on several effective thicknesses of ozone, the principal biologic endpoint being carcinogenesis in the skin of hairless mice. A xenon arc lamp is used to simulate sunlight and an optical filtration system is used to simulate changes in the stratospheric ozone content.

Major Findings: A significant body of data has been generated through the use of the light source, Cary spectroradiometer, Robertson-Berger UV meters, and Wang computer. An important feature of the system is the ability to compare ozone and glass filter optical properties, using one light source and one spectroradiometric detector system.

Results of the first biological experiment, recently completed, are presently being evaluated. Preliminary results indicate, as expected, an increase in carcinogenic activity with decreasing filter thickness (simulating decreased ozone thickness). The relationship is internally consistent and of the same order of magnitude whether expressed in terms of risk (time to 50% incidence) or yield (time to average yield of 1 tumor). Some inconsistencies were observed between nominal replications, and the basis for these differences is under investigation. A systematic variable external to the experimental design is implied by the high internal consistency within each replicate.

Detailed information is available concerning the amount and distribution of light supplied to each experimental group. These data cannot presently be used to generate a dose scale, however, since the appropriate weighting functions for the different spectral regions are not known. Instead we intend to use the data from the second and third experiments, in which the dose is varied while the spectra remain constant, to relate a given response to a "dose-equivalent". In this way we will back-calculate the "dose-equivalent" contribution of each spectral increment, and indirectly estimate an action spectrum. A provisional interpretation of the first experiment is that short wavelenght (< 300nm) may be more effective than predicted by model spectra currently in use.

Significance to Biomedical Research and the Program of the Institute: This project will yield scientific data relevant to the broad field of cancer biology and in addition provide data of immediate concern to the National Cancer Institute, to NASA, and to other agencies concerned with inadvertent modifications of stratospheric ozone. Assessment of the contribution of various wavebands of ultraviolet light to changes in the skin will play an important part in the understanding of naturally-occurring light-induced alterations. One immediate benefit of the study is the availability of a sophisticated spectrophotometric system for analysis of a variety of light sources. Several types of lamps have been analysed (e.g. filament lamps, arc lamps, flourescent lamps) and the data made available to others involved in photobiologic research.

Proposed Course: The first of the three major biology experiments began February 15, 1976. Replicate groups of this and the remaining experiments were phased into operation at approximately one month intervals.

Date Contract Initiated: February 15, 1974

Current Annual Level: $200,000

TEXAS, UNIVERSITY OF (NO1-CP-65846)

Title: The Biology of Neoplastic Liver Lesions in Mice

Contractor's Project Director: Dr. Frederick F. Becker

Project Officer (NCI): Dr. Richard A. Griesemer

Objectives: The major objective of this proposal was to develop objective methods of characterizing liver lesions which arise either spontaneously or by chemical induction in various inbred mouse strains.

Significance to Biomedical Research and the Program of the Institute: At present, the mouse is the major test animal for chemical carcinogens. A better interpretation of the measure of the level induced by these agents would yield more objective criteria for their designation as carcinogens.

Proposed Course: At present, many of the spontaneously arising tumors, as well as those induced by chemicals, are of unknown malignant potential; and, the part they play in malignant evolution is similarly uncertain. To avoid the subjective implications inherent in judgements made at the microscopic-pathologic level, a series of biological and biochemical measurements have been proposed which will be attempted to correlate with subsequent behavior of the lesions. Also, studies will be correlated with morphologic evaluation as well as the transplantability of these lesions - the only commonly accepted measure of malignancy. In addition, metastatic capability will also be examined.

Several modalities are to be studied. (1) It has been demonstrated that in selected instances, agglutination of suspended cells by plant lectins is characteristic only of malignant tumors and usually those of relatively aggressive nature. The mouse liver lesions will be suspended, and will be exposed to the lectins to determine if their biological potential correlates with this modality. (2) An increasing number of reports have been given evidence that characteristic patterns of chromatin associated protein exist in lesions that have been otherwise characterized as premalignant or malignant. The chromatin proteins of the mouse liver lesions will be similarly evaluated and attempts made to correlate these with the biological potential and sequential alterations studied by serial observation. (3) Evaluation of the sequential development and evolution of lesions throughout their course by repeated laparotomy, mapping of the distribution and nature of these lesions, and determination of eventual course within an individual mouse liver. (4) It has been demonstrated in other studies that the most characteristic association of alpha fetoprotein, and oncofetoprotein is with the appearance of malignant cells of the mouse liver. Recent reports from the laboratory have strengthened this association while casting some doubt upon the earlier induced waves of alpha fetoprotein associated with the administration of chemical carcinogens.

Attempts to determine by a sensitive radioimmunoassay for AFP the course and evolution of this protein during the development of spontaneous and induced lesions in the mouse will be made. Further, attempts to determine if the association between this protein and mouse liver lesions is invariably an indicator of malignancy will also be made. Such an association would make possible an absolute correlation without recourse to subjective evaluation.

Date Contract Initiated: September 30, 1976

Current Annual Level: $182,415

TRACOR JITCO, INC. (NO1-CP-43350)

Title: Bioassay Prime Contract

Contractor's Project Director: Dr. Marshall Steinberg

Project Officer (NCI): Dr. J. Fielding Douglas

Objectives: To provide scientific and management support to the Bioassay Operational Unit in the conduct of carcinogenesis bioassay testing of environmental chemicals. This entails the following: (1) maintain responsibility for accurate and timely performance of bioassay subcontracts under the prime; (2) develop plans, RFP's, advertise, evaluate and award new competitive and/or sole source subcontracts; (3) continue operation of a management system to coordinate and monitor the research conducted by the subcontractor; (4) propose and carry out scientific improvements and cost-saving/efficiency methods for the program, (5) coordinate resources and assure safety in the testing program; (6) purchase chemicals and obtain chemical analysis information on compounds to be tested or needed to continue tests in progress; (7) provide for data submission to the Carcinogenesis Bioassay Data System and assist in the preparation of final reports on the chemicals being tested; (8) establish and maintain a repository of residual bioassay materials; (9) evaluate, check and improve the quality of pathology services on the program - holding workshops as necessary to coordinate program diagnoses; (10) take action to dispose of chemicals residual to program needs; and (11) make a best effort in assuming other responsbilities as desired.

Major Findings: (1) Ten former NCI bioassay contracts were converted into Tracor Jitco subcontracts in previous fiscal years. These were Dow Chemical Company, Gulf South Research Institute, Hazelton Laboratories America, IIT Research Institute, Litton Bionetics, Mason Research Institute, Midwest Research Institute, Papanicolaou Cancer Research Institute, Southern Research Institute, and Stanford Research Institute. Since that time six new subcontracts have been awarded. The new awards were made to Industrial Bio-Test, Battelle Columbus Laboratories, Litton Bionetics, Mason Research, Gulf South Research, and Southern Research Institutes. A special study subcontract was awarded to the University of New Orleans and one to the Papanicolaou Cancer Research Institute. (2) Monitorship of the subcontracts was intensified. (3) A Management Plan and System for the Bioassay Operations has been continuously improved. (4) A list of prospective inhalation laboratories was developed from a public announcement. The qualified bidders' list for oral studies was expanded. (5) A revised competitive Basic Ordering Agreement (BOA) for initiation of testing of additional chemicals for future year's awards has been prepared; it will be released shortly. (6) Support activites for the NCI Working Groups on Chemical Selection, Experimental Design, Pathology and Data Evaluation were continued. (7) Coordination of animal and chemical resources was continued. (8) Intensive activity on a series of Technical Reports on chemicals tested was carried out and the first few reports produced; many other reports are in process now. (9) Actions were continued to maintain accurate and timely data input into the Carcinogenesis Bioassay Data System. (10) A repository for bioassay materials has been established. (11) Pathology workshops have been held and interface between and among pathologists improved. (12) Safety improvements have been made in several areas of the program; unannounced safety site visits are held routinely.

(13) Cage systems have been investigated in depth and improvements obtained from one manufacturer. (14) Subcontractor equipment and facilities are being steadily upgraded across the board.

Significance to Biomedical Research and the Program of the Institute: One of the primary factors basic to the understanding and prevention of cancer is the identification of carcinogenic chemicals in the environment of man. If "environment" is defined to include not only the atmosphere but also industry, transportation, clothing, housing, occupation, food, drugs, cosmetics, and any other entity with which man comes into contact, the number of suspect chemicals is very large. Thus, the number of chemicals which must be tested is probably large. Because of the nature of the bioassay, numerous animals must be utilized in testing each chemical over long periods of time. Extensive elements of data are produced which must be recorded accurately and interpreted expertly. This covergence of large-dimension factors results in high costs. In order to obtain the greatest value in knowledge with minimal expenditures of funds, it is essential that the program be managed carefully to ensure good science, expert use of manpower and funds, and high quality. A principal goal of the Prime Contractor, therefore, is to achieve the objectives of the NCI Bioassay Operational Unit with high scientific credibility at reasonable costs.

Proposed Course: It is intended to continue all on-going bioassays of chemicals to completion, with subsequent reporting of results in the NCI Carcinogenesis Technical Reports Series. The testing of a substantial number of additional chemicals will be commenced according to a schedule arranged in terms of availability of funds, animals, chemicals, and qualified laboratories. Improvements in the bioassay procedures are planned with respect to sensitivity of animal models to carcinogens and identification of alternative animal models; development of time-dose-response procedures which can be used to supplement or replace screening procedures; development of suitable inhalation test protocols; employment of biochemical procedures to give a better understanding of metabolic pathways and detoxication mechanisms involved during carcinogenesis tests; studying the design environment or host factors that may affect the outcome of bioassays, e.g., feed, water, biometry, consideration of ways and means to implement cost-savings and efficiency, by improvement in the design of laboratory facilities, streamlined methods of reporting pathology data, etc.

Date Contract Initiated: March 1, 1974

Current Annual Level: $10,000,000

Narrative statement of the 19 subcontracts to Tracor Jitco, Inc. are reported below.

1. Subcontract with Battelle Columbus Laboratories (76-34-106002)

Title: Carcinogenesis Bioassay

Subcontractor's Project Director: Dr. Arthur Peters

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. Thomas P. Griffin

Objectives: To investigate the carcinogenicity of nine aromatic azo dyes in Fischer 344 rats and B6C3F1 mice. The experimentally determined maximum tolerated doses will be incorporated into the ground diet and fed to groups of 50 male and 50 female rats and mice. Similar groups of animals will receive one-half the maximum tolerated dose. These chemicals will be administered to both species for 103 weeks followed by a one-week observation period. Adequate control animals will be maintained during the tests to permit statistical comparison.

Major Findings: Six of the nine dyes have completed prechronic study to determine maximum tolerated doses. Chronic carcinogenesis bioassay of C.I. Acid Orange 10, F.D. and C. Yellow No. 6, C.I. Acid Red No. 14, C.I. Disperse Yellow 3, D. and C. Red No. 9, and Solvent Yellow 14 have begun in both rats and mice; these studies are projected to continue for 104 weeks. Prechronic studies of Direct Blue 6, Direct Brown 95, and Direct Black 38 have just begun in both rodent species.

Significance to Biomedical Research and the Program of the Institute: A primary goal in the prevention of cancer consists of identifying potential cancer-causing agents. The aim of these carcinogenesis bioassays is to gather such data.

Proposed Course: At the end of each study, all animals that survive will be killed, necropsied, and the tissues examined histopathologically.

Date Contract Initiated: Tracor Jitco subcontract initiated February 23, 1976.

2. Subcontract with Dow Chemical Company (74-21-106002)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Subcontractor's Project Directors: Dr. S. D. Warner
Dr. C. G. Gerbig

Project Officer (Tracor Jitco): Mr. Donald J. Minnick

Objectives: To evaluate the potential carcinogenicity and chronic toxicity of five selected environmental chemicals used in commerce and industry by chronic bioassay in F344/CR rats and B6C3F1 mice.

Major Findings: The acute oral median lethal dose and subacute six weeks dietary studies were completed in rats and mice for 2-amino-5-nitro-thiazole, 1,3-dichloro-5,5-dimethylhydantoin, 3-nitropropionic acid,

proflavin dihydrochloride, N,N^1-dicyclohexyl thiourea and resorcine blue. In addition, acute and subacute studies on scopolamine hydrobromide were dropped from further testing and replaced with N,N^1-dicyclohexyl thiourea.

From the results of the subacute dietary studies, the maximum tolerated dose (MTD) was determined for each chemical in each species. The MTD and 1/2 MTD were used in the chronic rat studies of 110 weeks and mouse studies of 104 weeks duration. Each dose level consisted of 50 males and 50 females of the two rodent species. Due to lack of stability of 3-nitropropionic acid in the diet, it was administered five days per week via gavage. All other chemicals were administered via the diet which was prepared weekly or biweekly. N-2-fluorenylactamide was used as a positive control in these studies. No major problems developed during this contract period. All studies were in the chronic phase of testing at the beginning of the current contract.

During this contract all mouse studies were extended from 90 to 104 weeks of treatment. Mice that were administered 2-amino-5-nitrothiazole or 3-nitropropionic acid were subjected to gross postmortem examination in April 1975 and the rats receiving these chemicals were terminated in May 1975. Mice treated with N,N^1-dicyclohexyl thiourea, proflavine hydrochloride or 1,3-dichloro-5,5-dimethylhydantoin were terminated in June 1975 and the rats July 28 through August 5, 1975.

The overall survival was good in both the rat and mouse studies, the only exception being that of the positive control groups. All positive control rats either died or were killed prior to the scheduled necropsy. Hepatocellular carcinoma with metastasis to the lung was a constant finding in these groups. Survival was slightly better in the mice that received the positive control. The most common tumors observed in the mice were hepatocellular and bladder carcinoma, and hematopoietic neoplasms.

During October 1975, tissues and/or slides and blocks of control animals and those treated with proflavine hydrochloride were forwarded to Experimental Pathology Laboratories, Inc. (EPL) for pathologic evaluation. The control slides were returned for reading. Examination of slides of tissues from the remaining compounds were completed by Dr. J. L. Emerson currently with Abbott Laboratories with pathology narratives forwarded to CBDS for tabulation and analysis in February and March 1977. All residual tissues and/or slides and blocks were forwarded to the repository for storage in January 1977 and all residual chemicals were shipped to the chemical repository for final disposition in February 1977. All raw data for body weight, food consumption and tumor analysis has been submitted to CBDS for compilation and analysis. A final summary report of activities has been submitted to Tracor Jitco.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of determining potential carcinogenic hazards in the environment so that human use and exposure may be minimized. The aim of this program is to gather such data for a number of widely used materials.

Proposed Course: No additional work is anticipated. This contract is scheduled for termination on June 30, 1977.

Date Contract Initiated: Tracor Jitco subcontract initiated May 1, 1974; successor to NCI contract NO1-CP-23254, initiated June 12, 1972.

3. Subcontract with Experimental Pathology Laboratories (76-40-106002)

Title: Pathology Reporting of Assigned Bioassay Chemicals

Subcontractor's Project Director: Dr. William M. Busey

Project Officer (Tracor Jitco): Mr. George R. Pielmeier

Objectives: To assist Tracor Jitco, Inc. in the preparation of final reports of findings on Bioassay chemicals by the preparation of pathology reports on these chemicals.

Significance to Biomedical Research and the Program of the Institute: Within the Carcinogenesis Bioassay Program of the National Cancer Institute, there are numerous chemicals for which the animal phase of the testing has been completed and, in many cases, detailed histopathologic observations have been made, recorded and submitted to the Carcinogenesis Bioassay Data System (CBDS). In the past, the various subcontractors participating in the Program were not required to prepare final technical reports which would include the toxicologic information and a summary and interpretation of the pathology data. Because of these ambiguities in the early stages of the Program, the data derived from numerous chemicals have not been evaluated and interpreted. Many of these chemicals on which testing has been completed are of significant economic importance to their manufacturer and to the public in general.

Proposed Course: The pathology reports will be prepared and submitted at the rate of two chemicals per week.

Date Contract Initiated: Tracor Jitco subcontract initiated November 8, 1976

4. Subcontract with Gulf South Research Institute (74-27-106002)

Title: Carcinogenesis Bioassay of Chlorinated Hydrocarbons, Organophosphates and Miscellaneous Pesticides

Subcontractor's Project Director: Mr. Ralph J. Wheeler

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. Thomas Griffin

Objectives: To evaluate the carcinogenicity of 27 chlorinated hydrocarbons, organophosphastes, and miscellaneous pesticides. The three positive control chemicals included in the study were 2-AAF, safrole, and amitrole.

The animals in the first group (Series I) were fed the pesticides for 80 weeks. Both sexes of Osborne-Mendel rats and B6C3F1 mice at two dose levels (estimated MTD and 1/2 MTD), using a group size of 50, were employed. Mice were sacrificed at 90 weeks and rats at 110 weeks. A similar protocol was used for the second group (Series II), except that F344 rats and B6C3F1 mice are exposed for 103 weeks, after which time they are sacrificed.

Major Findings: Series I compounds were put on test during the period of August 1971 through September 1972 and taken off test during the period of August 1973 through October 1974. The estimated maximum tolerated dose (MTD) was determined by six-week feeding studies. In no case was the dose level tolerated in the chronic phase of the bioassay, and all doses had to be reduced during the test because of toxic effects. In some cases the final MTD was only 10% of the initial MTD. However, it is felt that initial use of 25% of the MTD as determined by six-week studies would have been satisfactory. Animals from the Series I chronic tests have now been sacrificed and their tissues have been evaluated by the pathologists and summary reports are being prepared on the findings by Tracor Jitco staff.

Series II compounds were put on 104 week chronic test, using Fischer 344 rats and B6C3F1 mice, during the period December 1974 through December 1975. Animals from the first four compounds have been sacrificed and their tissues are being studied by pathology.

Significance to Biomedical Research and the Program of the Institute: Many of the pesticides under study have widespread usage resulting in extensive human exposure. The chemicals selected for testing have either been tested inadequately for carcinogenicity in the past or the results of such tests have been equivocal. These extensive experiments are designed to help resolve these issues.

Proposed Course: Pathology will be completed during the year and reports prepared on the Series II pesticides. The last animals are scheduled for sacrifice in December 1977.

Date Contract Initiated: Tracor Jitco subcontract initiated November 1, 1974; successor to NCI contract NO1-CP-43267 initiated June 15, 1970.

5. Subcontract with Gulf South Research Institute (76-36-106002)

Title: Bioassay of Environmental Chemicals

Subcontractor's Project Director: Mr. Ralph J. Wheeler

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. Thomas Griffin

Objectives: To evaluate the carcinogenicity of a series of eight low molecular weight chlorinated hydrocarbons. The compounds, methylene chloride, methyl chloroform, perchlorethylene, cis-1,2-dichloroethylene, trans-1, 2-dichloroethylene, pentachloroethane, tetrachloroethane and vinylidene chloride are to be administered by gavage in corn oil to Fischer 344 rats and B6C3F1 mice. In addition, perchloroethylene will be studied in Long-Evans, Wistar and Sherman rats. A colony of Sherman rats is being bred to provide the test animals.

Major Findings: Subchronic tests have been completed on methylene chloride, vinylidene chloride, pentachloroethane and tetrachloroethane. Results of the last two are not avaiable. Results on methylene chloride were not sufficient to establish an MTD due to lack of toxicity. Vinylidene chloride proved hepatotoxic down to the lowest level tested 5 mg/kg. Recommendations are that both subchronics be rerun at low levels.

Significance to Biomedical Research and the Program of the Institute: This is a series of one and two carbon halogenated hydrocarbons, some of which have been tested by other routes and found to be carcinogenic. Several are in widespread use as degreasers and dry cleaning solvents, while one is used to extract caffeine from coffee. This will complete this series of compounds.

Proposed Course: Chronic tests are scheduled to begin in April 1977 on two compounds while subchronics are being completed on the others. The last compound of the series should go on chronic test in February 1978.

Date Contract Initiated: Tracor Jitoc subcontract initiated April, 1976

6. Subcontract with Hazelton Laboratories (74-28-106002)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Subcontractor's Project Directors: Dr. Marcelina B. Powers
Dr. Richard W. Voelker

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. Jane F. Robens

Objectives: To determine the carcinogenicity of selected pesticides and other environmental chemicals in Osborne-Mendel and Fischer 344 rats, B6C3F1 and ICR-Swiss mice and Syrian Golden hamsters. Three series of environmental chemicals are being investigated. Series A consists of 117 perticides administered in the feed to rats and mice for 78 weeks, with observation periods of 32 weeks in rats and 12 weeks in mice. Series B consists of 18 halogenated industrial chemicals administered by oral

intubation to rats and mice for 78 weeks with a 32-week observation in rats and 12-week observation in mice. Series C consists of 11 chemicals administered by various routes, e.g., in the feed, by oral gavage, in the drinking water, by application to the skin, and by inhalation, for 103 weeks followed by a one-week observation period. Fischer 344 rats and B6C3F1 mice are used for chemicals administered orally and by inhalation, except for the use of the Syrian Golden hamster in the phenol inhalation study. ICR Swiss mice are used for the cutaneous application studies.

Major Findings: Of the 46 chemicals under study, bioassay including histopathology has been completed on 35 chemicals (Series A and B). Reports have been published on three chemicals - trichloroethylene (TCE), chloroform, and 1,1,1-trichloroethane (methylchloroform). Definitive histopathologic diagnosis with statistical confirmation has been made on eight additional chemicals that have completed bioassay. Three chemicals, ethylene dibromide (EDB), 1,2-dibromo-3-chloropropane (DBCP), and chloroform produced effects suggestive of carcinogenicity in both rats and mice. EDB and DBCP were associated with squamous cell carcinoma of the stomach with metastases. DBCP also induced an increased incidence of mammary carcinoma in female rats. Chloroform induced kidney tumors in male rats and hepatocellular carcinoma in mice. TCE, chlorobenzilate, tetrachloroethylene, 1,1,2-trichloroethane, and chloropicrin did not appear to be carcinogenic in rats, but appeared to be carcinogenic in mice inducing significantly increased incidences of hepatocellular carcinomas in this species. The chemicals PCNB, Zectran, Thiodan, and methylchloroform did not appear to be carcinogenic to rats or mice under the test conditions of these bioassays.

Of the 11 chemicals in Series C, chronic oral studies have been terminated in rats and mice for five chemicals. Chronic inhalation studies for EDB and DBCP are repeated dermal studies for selenium disulfide and Selsun® are in progress. The prechronic toxixity studies on phenol and "promoted" phenol (with benzpyrene) have been completed and the exposure levels established for the planned chronic studies.

Significance to Biomedical Research and the Program of the Institute: The chemicals under study have widespread usage and in many instances are components of products to which the human population is extensively exposed. If any of these chemicals are found to be carcinogenic, measures to control and minimize human exposure and to assess possible significance of human exposure would be appropriate. Information gathered from these studies will also be valuable in establishing precautionary measures for handling and using these chemicals and for planning future investigations.

Proposed Course: The chronic studies currently in progress or planned for the remaining chemicals will be completed accoring to the established schedules and procedures and reports will be prepared on these chemicals.

Date Contract Initiated: Tracor Jitco subcontract initiated February 15, 1975; successor to NCI contract NO1-CP-43245 initiated December 1, 1970.

7. Subcontract with IIT Research Institute (74-22-106002)

Title: Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals

Subcontractor's Project Director: Dr. Patricia Holmes

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. Steve Olin

Objectives: To determine the carcinogenicity and/or co-carcinogenicity of a series of chlorinated dibenzodioxins and related compounds by oral administration to mice and rats and by application to the skin of mice.

Major Findings: Prechronic oral intubation studies of tetra- and hexachlorodibenzodioxins (TCDD and HCDD) established the toxic effects and cumulative toxicity of these compounds. The cumulative toxicity in the subchronic studies precluded determination of the MTD in certain experiments. Doses for chronic administration of TCDD and HCDD were chosen after consideration of the delayed cumulative toxicity found in the prechronic studies. TCDD and HCDD were administered by oral intubation to Osborn-Mendel rats and to B6C3F1 mice for two years; these experiments have just ended and histopathology is in progress. Both compounds are also being applied to the skin of Swiss-Webster mice; these experiments are scheduled to end in November 1977. Acute and subchronic studies have been carried out with Trichlorodibenzodioxins (Tri CDD). This compound has proved to be considerably less toxic than either TCDD or HCDD, and because of this, the decision has been made not to carry out chronic studies with Tri CDD. Chronic bioassays of unsubstituted dibenzodioxin, dichlorodibenzodioxin, octachlorodibenzodioxin (the "non-toxic" dioxins) and dioxane have been completed. Reports are in progress for all of these compounds with the exception of octachlorodibenzodioxin.

Significance to Biomedical Research and the Program of the Institute: Some chlorinated dibenzodioxins are extraordinarily toxic contaminants in certain perticides and other environmental chemicals. In addition to their extreme toxicity, they are thought to be highly stable under environmental conditions and may accumulate in the food chain. They have been tested for acute toxicity and teratogenicity but have not been examined for carcinogenicity. This program is designed to accomplish the latter and is part of the NCI program of testing chemicals to which man is exposed for carcinogenicity.

Proposed Course: Histopathology will be completed and reports generated for the chronic oral studies with HCDD and TCDD. The same will be done for skin-painting experiments when they are terminated in November 1977.

Date Contract Initiated: Tracor Jitco subcontract initiated May 1, 1974; successor to NCI contract NO1-CP-12338 initiated June 25, 1971

8. Subcontract with Industrial Bio-Test (76-33-106002)

Title: Carcinogenesis Bioassay

Subcontractor's Project Director: Mr. Dennis Lindberg

Project Officer (Tracor Jitco): Dr. Steve Olin

Objectives: To investigate the carcinogenicity of selected compounds in Fischer rats, B6C3F1 mice, and Syrian Golden hamsters. The experimentally determined maximum tolerated concentrations will be administered by inhalation to groups of 50 male and 50 female rats, mice, and hamsters. Similar groups of animals will receive one-half the maximum tolerated concentration. These chemicals will be administered to these species for 103 weeks followed by a one week observation period. Adequate control animals will be maintained during the tests to permit statistical comparison.

Major Findings: No major findings can be reported at this date, since only acute, repeated dose, and subchronic studies have been initiated.

Significance to Biomedical Research and the Program of the Institute: A primary goal in the prevention of cancer consists of identifying potential cancer causing agents. The aim of these carcinogenesis bioassays is to gather such data.

Proposed Course: At the end of each of the studies completed so far all surviving animals have been killed, necropsied, and the tissue examined histopathologically when deemed necessary by the pathologist. This applied only to acute and repeated dose studies.

Three compounds are currently in the ninety-day portion of the program. Chronic studies are due to start in October.

Date Contract Initiated: Tracor Jitco subcontract initiated February 23, 1976

9. Subcontract with Litton Bionetics, Inc. (74-25-106002)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Subcontractor's Project Directors: Mr. Robert A. Stewart
Mr. Stephen L. Johnson

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. William MacDonald

Objectives: This program is designed to investigate the carcinogenicity and chronic toxicity of selected chemicals in Fischer 344 rats and B6C3F1 mice. The maximum tolerated doses experimentally determined for each chemical are incorporated into ground diet or administered by gastric intubation or intraperitoneally to groups of 50 male and 50 female rats and mice. Similar groups of animals will receive one-half the maximum tolerated dose. Prior to December 1, 1974, experimental substances were administered for a period of 18 months, with a six month observation period in rats and a three month observation period in mice. After this date, both species were dosed for 103 weeks with an additional one week observation period. At the end of the study, all animals are subjected to necropsy and histologic examination.

Major Findings: Of the 42 chemicals under study, histopathologic examination has been completed on the mice and rats for all but four compounds. Summary reports with accompanying pathology narratives are in progress.

Significance to Biomedical Research and the Program of the Institute: By identifying potentially hazardous substances present in the environment, using the mouse and rat as a model, it may be possible to minimize man's exposure by prohibiting or restricting the use of these agents or modifying the method in which they are processed and handled.

Proposed Course: To complete summary reports with accompanying pathology narratives on the chemical compounds under study.

Date Contract Initiated: Tracor Jitco subcontract initiated August 1, 1974; successor to NCI contract NO1-CP-43349 initiated May 13, 1971

10. Subcontract with Litton Bionetics, Inc. (75-31-106002)

Title: Carcinogenesis of Bioassay Monomers

Subcontractor's Project Director: Dr. Elliot B. Gordon

Project Officers (Tracor Jitco): Dr. Marshall Steinberg
Dr. William MacDonald

Objectives: To investigate the carcinogenicity of eight monomeric compounds and one industrial solvent. Oral administration either by dosed feed or gavage will be used for the monomers; skin painting of Swiss mice will be used for the industrial solvent (ethylene chlorohydrin).

Maximum tolerated doses and backup dose levels will be administered to groups of 50 Fischer rats and B6C3F1 mice of each sex. Swiss mice, only, will be used to evaluate ethylene chlorohydrin. Each compound will be administered for 103 weeks followed by a week of observation.

Major Findings: Four dosed feed chronic studies were initiated in January and February, 1977 (caprolactam, bisphenol A, aminoundecanoic acid and diaminodichlorobenzene). Subchronic studies are in progress on three gavage compounds (toluene diisocyanate, diallylphthalate and diphenyl methane diisocyanate) and one dosed feed compound (melamine). The skin painting studies are beginning the subchronic testing phase.

Significance to Biomedical Research and the Program of the Institute: A primary goal in the prevention of cancer consists of identifying potential cancer causing agents. The aim of these carcinogenesis bioassays is to gather such data.

Proposed Course: At the end of each study all animals that survive will be killed, necropsied, and the tissues examined histopathologically.

Date Contract Initiated: Tracor Jitco subcontract initiated November 3, 1975

11. Subcontract with Mason Research Institute (76-32-106002)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Subcontractor's Project Director: Dr. John Landon

Project Officer (Tracor Jitco): Dr. William MacDonald

Objectives: To evaluate the carcinogenicity of nine environmental chemicals. Matched diet controls will be run. These compounds are the fifth series.

The series V chemicals are being mixed in the diet at two levels, the estimated maximum concentration consistent with prolonged survival of the animals and one-half of this concentration. Food containing these levels of each chemical is being fed for 24 months in groups of Fischer 344 rats and B6C3F1 mice. Groups consist of 50 males and 50 females.

All animals are being observed twice daily for signs of chemical toxicity and tumor development. In addition, body weight and food consumption is being measured at monthly intervals. Animals which are moribund or which die and all animals which are sacrificed receive extensive gross and microscopic examination, including a detailed examination of gross lesions and tissue masses and all major organs.

Major Findings: Two Series V compounds along with respective controls, are presently in chronic phases of study. The remaining Series V compounds should enter chronic study by October 1977.

Significance to Biomedical Research and the Program of the Institute: One of the functions of the Carcinogenesis Program is to evaluate the potential carcinogenic risk that is represented by chronic exposure to environmental chemicals. This contract will be significant in identifying and minimizing potentially hazardous substances to which man is exposed.

Proposed Course: Bioassay experiments will be continued through the chronic tests, sacrifice, necropsy, pathology, data reporting, and preparation of Techincal Reports.

Date Contract Initiated: Tracor Jitco subcontract initiated April 30, 1976

12. Subcontract with Mason Research Institute (74-29-106002)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Subcontractor's Project Director: Dr. John Landon

Project Officer (Tracor Jitco): Dr. William MacDonald

Objectives: To evaluate the carcinogenicity of 54 environmental chemicals or combinations of chemicals, grouped into four series. For positive control, chemicals included in the studies are 2-AAF and 3-amino-1,2,4-triazole. Negative and vehicle (acetone and corn oil) controls also are run.

The Series III and IV chemicals are being mixed in the diet at two levels, the estimated maximum concentration consistent with prolonged survival of the animals and one-half of this concentration. Food containing these levels of each chemical is being fed for 24 months in groups of Fischer 344 rats and C57BL/6 or B6C3F1 mice. Groups consist of 50 males and 50 females.

All animals are being observed twice daily for signs of chemical toxicity and tumor development. In addition, body weight and food consumption is being measured at monthly intervals. Animals which are moribund or which die and all animals which are sacrificed receive extensive gross and microscopic examination, including a detailed examination of gross lesions and tissue masses and all major organs.

Major Findings: All animals treated with chemicals of Series I, II and III have been necropsied. Histopathologic evaluation on Series I and II has been completed. Series III compounds are near completion, and a Series IV compound is undergoing evaluation. Two Series IV compounds along with respective controls, are presently in chronic phases of study. The one remaining Series IV compound should enter chronic study by mid-summer 1977.

Significance to Biomedical Research and the Program of the Institute: One of the functions of the Carcinogenesis Program is to evaluate the potential carcinogenic risk that is represented by chronic exposure to environmental chemicals. This contract will be significant in identifying and minimizing potentially hazardous substances to which man is exposed.

Proposed Course: Bioassay experiments will be continued through the chronic tests, sacrifice, necropsy, pathology, data reporting, and preparation of Technical Reports.

Date Contract Initiated: Tracor Jitco subcontract initiated December 29, 1974; successor to NCI contract NO1-CP-43297 initiated May 1, 1971

13. Subcontract with Midwest Research Institute (74-24-106002)

Title: Analytical Chemistry Resource

Subcontractor's Project Director: Dr. Evelyn Murrill

Project Officer (Tracor Jitco): Dr. Marshall Steinberg
Dr. Stephen S. Olin

Objectives: This program serves as an analytical resource for the Prime Contract. Specific tasks include analysis of chemicals for identity, assay, and stability; formulation of protocols for chemical mixes; analysis of feed samples for toxic components; and analysis of dose-feed samples. Special tasks include isolation and identification of impurities and other analytical problems.

Major Findings: Since June 1972, studies have been completed and reported on 433 batches of chemicals (248 different chemical substances). Sixty-nine have been analyzed in detail to identify and quantitate minor impurities, and 187 chemical mixes have been analyzed for stability or homogeneity. Routine characterization studies include chromatography (TLC, GC, LPLC, etc.), elemental analysis, water analysis, and other assay methods, depending on the chemical (titration, voltammetry, mass spectrometry). Physical data include transition data (melting point, boiling point), refractive index, optical rotation, and spectral data (infrared, ultraviolet, visible, mass and NMR). Chemical/feed mixtures have been analyzed at zero time and after storage intervals by vapor phase and high-pressure liquid chromatography, voltammetry, or spectophotometric techniques. Preparative high-pressure liquid chromatography and GC/MS have been used with some of the techniques listed under routine analysis for solving impurity and purification problems.

Significance to Biomedical Research and the Program of the Institute: The literature of carcinogenesis bioassay studies contains many examples of experiments which cannot be meaningfully interpreted because the task substances have not been adequately characterized chemically. When such an experiment results in low tumor incidence, for example, the question will always remain as to whether the tumors observed might not have arisen from small concentrations of highly potent impurities, rather than from the allegedly weakly potent nominal ingredient. Other questions of a chemical nature which are central to the interpretation of bioassay data include

such problems as decomposition of the chemical in feed or intubation mixtures and levels of mycotoxins or pesticides in food and bedding. For these reasons, it is essential that the Carcinogenesis Program have available to it a facility capable of characterizing those materials entered into the testing program as well as determining the effectiveness of administration of these substances to the test animals.

Proposed Course: To continue to serve as an analytical resource for the bioassay program by homogenization and analysis of bulk chemicals, analysis of vehicles, stability analysis of the bulk chemical and of chemical/vehicle mixtures, and by the preparation of protocols for obtaining homogeneous chemical/vehicle mixtures.

Date Contract Initiated: Tracor Jitco subcontract initiated July 1, 1974; successor to NCI contract NO1-CP-23270 initiated June 28, 1972

14. Subcontract with Papanicolaou Cancer Research Institute (74-30-106002)

Title: Comparative Study of the Responsiveness of Six Inbred Strains of Rats to a Known Carcinogen

Subcontractor's Project Director: Dr. Wilhelmina F. Dunning

Project Officer (Tracor Jitco): Mr. Donald Minnick

Objectives: To determine the potential usefulness of several inbred strains of rats for carcinogenicity bioassay by comparing their responsiveness to a known chemical carcinogen.

Major Findings: Comparative chronic tests are completed with the chemical N-hydroxy-N-2-fluorenylacetamide in six inbred strains of rats. These include the Fischer line 344, A x C line 9935 Irish, August line 990, Marshall line 520, S x F line 40814 and Zimmerman line 61.

A total of 697 rats were fed N-hydroxy-N-2-fluorenylacetamide on a chronic schedule. The females received 10.1 and 5.0 mg/kg by incubation on a 5-day-a-week schedule for six months. The males were given 5.0 and 2.5 mg/kg for a similar time.

The necropsy records are complete for all of the rats that survived at least six months of chronic feeding. A total of 312 induced neoplasms have been described, of which 310 have been confirmed and classified by microscopic examination. The most frequent site of neoplastic changes was the liver with 129 tumors, of which 127 have been classified as malignant hepatomas or adenocarcinomas with a few cholangiomas. Multiple liver tumors were frequent. The liver tumors occurred in rats of each strain, more frequently in males than females at the higher dose levels. The squamous division of the stomach was the next most frequent site of neoplastic change. In this organ 107 neoplasms have been described and

all have been confirmed to be squamous cell carcinomas. About 35 percent of these tumors were observed in rats of the August line 990. In addition, 23 A x C line 9935 and S x F line 40814 rats were observed with squamous cell carcinomas of the face, orbit, or ear canal, and 19 with carcinomas of the urinary bladder. Tumors of the mammary gland (14), uterus (6), ovary (2), spleen (3), lung (2), and mesenteric lymph node (13) were less frequent.

Approximately 100 rats(16 of each strain) served as vehicle controls and another group of 100 as untreated controls. Only twelve neoplasms were observed in these controls, none in the liver or stomach.

Significance to Biomedical Research and the Program of the Institute: As part of the Bioassay Program, compounds are tested in only one or two different rat strains. As an outcome of this study, more suitable strains of rats than those presently in use may be found.

Proposed Course: The findings are being analyzed and will be published shortly.

Date Contract Initiated: Tracor Jitco subcontract initiated February 10, 1975; successor to NCI contract NO1-CP-23253 initiated June 26, 1972

15. Subcontract with Papanicolaou Cancer Research Institute (77-44-106002)

Title: Comparative Study of the Responsiveness of Four Inbred Rat Strains to Trichloroethylene

Subcontractor's Project Director: Dr. Norman H. Altman

Project Officer (Tracor Jitco): Mr. Donald Minnick

Objectives: To determine the relative sensitivity of Marshall 520, ACI, August 990 and Fischer 344 rats to trichloroethylene.

Significance to Biomedical Research and the Program of the Institute: At the present time, the only acceptable procedure to determine if a chemical poses a carcinogenic risk to humans is to test that chemical in rats or mice. The selection of the most appropriate strain of rodent for carcinogenesis bioassay is therefore vitally important to the success of the National Cancer Institute's Carcinogenesis Program. This project should provide reliable information as to the relative strain sensitivity of the four inbred strains tested to a known carcinogen.

Proposed Course: Subchronic studies are in progress and it is anticipated that the chronic studies will begin in September 1977.

Date Contract Initiated: Tracor Jitco subcontract initiated February 15, 1977

16. Subcontract with Southern Research Institute (74-26-106002)

Title: Carcinogenicity Studies of Chemotherapeutic Agents and Related Chemicals

Subcontractor's Project Directors: Dr. D.P. Griswold
Dr. J.C. Peckham
Dr. J.D. Prejean

Project Officer (Tracor Jitco): Dr. Thomas P. Griffin

Objectives: To evaluate the potential carcinogenicity of chemotherapeutic agents and related chemicals in rats and mice following long-term exposure.

A variety of protocols have been used in evaluating the compounds assigned to this subcontract. This first protocol, covering 43 compounds or combinations of compounds, required that the agents be administered by intraperitoneal injection or gavage three times a week for six months at a maximum tolerated dose (MTD) and 1/2 MTD to groups of 25 male and 25 female rats and mice. A 12-month observation period preceded terminal sacrifice. Control groups were not started concurrently with treated groups.

The second protocol, covering 28 compounds, required that the agents be administered by intraperitoneal injection (3x/week), gavage (3x/week) or dosed-feed (5x/week) at an MTD and 1/2 MTD to groups of 35 male and 35 female rats and mice. Injection and gavage compounds were administered for 12 months and feeding compounds for 18. A six month observation period preceded terminal sacrifice. Control groups were started concurrently with each compound. In November 1974, all studies still in the treatment stage were altered to include 103 weeks of treatment followed by a one-week observation period prior to terminal sacrifice.

The final protocol utilized follows the Guidelines for Carcinogen Bioassay in Small Rodents (NCI Technical Report Series No. 1) except that treatment is three times per week for those agents administered by intraperitoneal injection.

Major Findings: Results of the studies on the 43 compounds or combinations of compounds evaluated under protocol No. 1 are currently being reviewed and a single summary report is scheduled for completion prior to November 1977.

Results of the studies on the 28 compounds evaluated under protocol No. 2 are currently being assembled as technical reports for submission to Tracor Jitco. Complete reports have been submitted on eight compounds - dimethane sulfonate (C01547), isophosphamide (C01683), 4,4'-sulfonyldianiline (C01718), 4,4'-thiodianiline (C01707), procarbazine (C01810), o-anthranilic acid (C01730), pyrazinamide (C01685), and l-tryptophane (C01729).

Results of the studies on the four compounds evaluated under protocol No. 3 are not complete. The chronic bioassays on Adriamycin and hexamethylmelamine have been completed and histopathology is underway. Reserpine and Cytembena chronic bioassays are still in progress.

Significance to Biomedical Research and to the Program of the Institute: Results of these studies will provide information as to the carcinogenicity of each compound in experimental animals which can be used as a guideline for evaluating their potential risk to man.

Proposed Course: To continue the two chronic bioassays in progress and to complete final reports on all compounds evaluated under protocols 1 and 2, and on the two compounds evaluated under protocol 3 by September 1, 1977.

Date Subcontract Initiated: Tracor Jitco subcontract initiated October 1, 1974; successor to NCI contract NO1-CP-33214 initiated June 24, 1968

17. Subcontract with Southern Research Institute (76-35-106002)

Title: Carcinogenesis Bioassay

Subcontractor's Project Directors: Dr. D.P. Griswold
Dr. J.D. Prejean

Project Officer (Tracor Jitco): Dr. Thomas P. Griffin

Objectives: To investigate the carcinogenicity of selected chemicals in rats and mice. The protocol consists of a series of prechronic studies to determine a maximum tolerated dose (MTD) for use in the chronic study. The MTD and 1/2 MTD will be administered to both species for 103 weeks followed by a one-week observation period. Appropriate control groups will be included. All groups will consist of 50 animals.

Major Findings: The acute and repeated-dose phases of the study have been completed for all nine compounds being evaluated under this subcontract. MTD's have been determined for mannitol (rats and mice), ziram (mice only), and eugenol (rats and mice).

Significance to Biomedical Research and to the Program of the Institute: Results of these studies will provide information as to the carcinogenicity of each compound in experimental animals which can be used as a guideline for evaluating their potential hazard to man.

Proposed Course: To complete all prechronic evaluations and begin chronic studies on all available compounds.

Date Subcontract Initiated: Tracor Jitco subcontract initiated May 1, 1976

18. Subcontract with Stanford Research Institute (74-23-106002)

Title: Combined Effects of Chemical Carcinogens and Other Chemicals

Subcontractor's Project Director: Dr. David C.L. Jones

Project Officer (Tracor Jitco): Dr. William MacDonald

Objectives: To investigate (1) the effect of mixtures of carcinogenic and noncarcinogenic chemicals on the induction of tumors when introduced into rodents by oral administration, (2) the interactions among groups of chemicals in acute and subacute toxicity experiments, and (3) the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the groups.

Major Findings: Histologic evalation has been completed for all compounds and paired combinations of compounds within the four chemical groups (Group 1 - N-methyl-N'-nitro-N-nitrosoguanidine, N-butanol-N-butylnitrosamine, nitrilotriacetic acid, dipentylnitrosamine; Group 2 - alfatoxin B_1, cycad flour, lasiocarpine, dipentylnitrosamine; Group 3 - aflatoxin B_1, N-butanol-n-butylnitrosamine, lead acetate, thiouracil; Group 4 - alfatoxin B_1, dieldrin, hexachlorophene, Arochlor 1254). All data have been submitted to CBDS. A final report covering effort under this contract up to the analysis of the histologic findings is in preparation. Statistical evaluation of the histologic data is in process under separate contract.

Significance to Biomedical Research and the Program of the Institute: A major portion of the resources of the National Cancer Institute's Carcinogenesis Program is devoted to testing environmental chemicals for carcinogenicity. In these tests, the experimental protocols generally attempt to expose animals to one chemical while minimizing exposure to other chemicals. There are two problems involved in this approach. First, in the human environment, men are exposed to multiple chemicals simultaneously and sequentially rather than to a single chemical. Second, resources are available to test only a small portion of the chemicals in our environment if they are tested singly. Newer chemicals are generated faster than the available resources are capable of testing each one by a protocol considered acceptable. This project is the beginning of a program that will investigate how mixtures of carcinogenic and noncarcinogenic chemicals interact to affect the induction of tumors and also to investigate the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the group. The biological studies from this contract will be analyzed by mathematical statisticians working on a separate contract with the NCI to determine whether consistent mathematical models of interactions can be developed.

Proposed Course: Statistical evaluation of the data will be completed and a final report prepared under separate contract.

Date Contract Initiated: Tracor Jitco subcontract initiated June 1, 1974; successor to NCI contract NO1-CP-43272 initiated June 24, 1971

19. Subcontract with the University of New Orleans (76-37-106002)

Title: High Resolution Gas Chromatography-Mass Spectrometry-Computer Analysis of Volatile Organics Found in Blood and Liver Tissues of Rats

Subcontractor's Project Director: Dr. John Laseter

Project Officer (Tracor Jitco): Dr. Neil Jurinski

Objectives: This study required the profiling and identifying of volatile components of male Fischer 344 rat tissue and blood of animals on test by gavage with trichloroethylene administered in corn oil. GC-MS-COMP methodology was utilized with the goal of aiding in the determination of the prospective maximum tolerated dose for trichloroethylene in this strain of rats.

Major Findings: Whole blood from animals sacrificed after ten days of treatment was found to be a more preferable medium for analysis than plasma. The effects of storage of frozen whole blood for up to nineteen days on the GC profiles was studied. Profiles were found to be reasonably consistent for this period of storage. Liver GC profiles appeared to show more variability upon 18 days of storage than the blood samples. Samples from the thirty-one day and ninety-three day sacrifices are in the process of analysis and evaluation.

Significance to Biomedical Research and the Program of the Institute: (1) This project will assist in determining the absolute concentration of compound in body fluids and tissue as compared with the administered dose. This is pertinent to the setting of environmental action levels. (2) This project will assist in establishing metabolic changes from the GC profiles at various exposure concentrations. (3) Bioaccumulation of impurities can be mointored.

Proposed Course: (1) It is proposed that the absolute concentration of compounds under test in the Bioassay Program in body fluids and tissues of animals exposed by gavage be compared with those exposed by inhalation, and further, that different vehicles for gavage be studied. It should be noted that corn oil might not be the optimal medium, as oils are routinely used to retard gastrointestinal absorption in cases of acute poisoning of TCE. Therefore, different vehicles for gavaging should also be incorporated into the study. (2) The project should be expanded to include other halogenated hydrocarbons. Also, new analytical techniques maximizing recovery should be incorporated into future studies.

Date Contract Initiated: Tracor Jitco subcontract initiated August 31, 1976

CONTRACT NARRATIVES

BIOASSAY OPERATIONAL UNIT
In Vitro Program

July 1, 1976 through September 30, 1977

AMERICAN HEALTH FOUNDATION (NO1-CP-55705)

Title: Development of Detailed Methods and Protocols for Carcinogen Screening Using Cell Culture Assays

Contractor's Project Director: Dr. Gary M. Williams

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To validate rat liver epithelial cell lines for the purpose of chemical carcinogen screening and to determine the response of these lines to carcinogens, procarcinogens and noncarcinogenic analogs in order to develop methods and procedures for such screening.

Major Findings: The study of malignant transformation in long-term cultures of rat liver epithelial cells has focused on identifying quantitative in vitro markers of transformation and establishing the correlation of these markers with tumorigenicity. A good correlation was found between growth in soft agar and malignancy while no correlation existed between the production of plasminogen activating factor and either growth in soft agar or tumorigenicity in nude mice. Continuous treatment of adult rat liver cultures for 4 or 8 weeks with procarcinogens at 10^{-6}M has been found to induce increases in the colony forming efficiency in soft agar by aflatoxin B_1 and dimethylnitrosamine.

As an adjunct to the transformation studies, other rapid indicators of carcinogenic activity such as mutagenesis and DNA repair are under study.

The mutagenesis studies utilize resistance to 8-azaguanine as a means of selecting hypoxanthine-guanine phosphoribosyl transferase-deficient mutants. This procedure worked well with the liver epithelial lines and it was found that various treatments with aflatoxin B_1, 7,12-dimethylbenzanthracene, dimethylnitrosamine, N-2fluorenylacetamide, and 3'-methyl-4-dimethylamino-azobenzene increased the frequency of mutants.

Primary rat liver cell cultures were found to be suitable for the autoradiographic determination of DNA repair as unscheduled DNA synthesis (UDS). UDS was elicited by direct acting carcinogens and procarcinogens requiring metabolic activation. It was dependent upon dose and duration of treatment and was not inhibited by hydroxyurea. Evaluation of the sensitivity of the system has been concentrated on classes of procarcinogens for which there are structural analogs varying in carcinogenic potency including

noncarcinogenicity. A brief outline of the results is as follows: In the series of mycotoxins, positive results were obtained with aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, and negative, with aflatoxin G_2; in the polycyclic aromatic hydrocarbon series, positive with 3-methylcholanthrene, 7,12-dimethylbenzanthracene, and negative with benzanthracene and anthracene; in the nitrosamine series, positive with diethylnitrosamine and dimethylnitrosamine and negative with dimethylformamide; in the aromatic amine series, positive with N-2-fluorenylacetamide, 3-methyl-2-naphthylamine, 2'3-dimethyl-4-aminobiphenyl, 4-aminobiphenyl and questionably positive with N-4-fluorenylacetamide, negative with biphenyl; in the aminoazo dye series with 3'-methyl-4-dimethylaminoazobenzene, 4'-methyl-4-dimethylaminoazobenzene, 2-methyl-4-dimethylaminoazobenzene and 4-aminoazobenzene.

Significance to Biomedical Research and the Program of the Institute: The studies on epithelial cell lines are important to the analysis of chemical carcinogenesis in epithelial tissues which are the site of the great majority of human cancers. Furthermore, the induction of transformed properties and mutations in these lines by a variety of procarcinogens indicates that they may be useful in rapid screens for chemical carcinogens. The induction of DNA repair in primary liver cultures by a wide variety of procarcinogens provides strong evidence that this system has great potential as an in vitro screen.

Proposed Course: (1) The identification and validation of markers for transformation in the long-term cultures of rat liver epithelial cells will be completed; (2) The sensitivity of selected markers of transformation for detecting transformation by carcinogens will be determined; (3) The mutagenesis and DNA repair assays will be further developed as adjuncts to the transformation studies.

Date Contract Initiated: June 30, 1975

Current Annual Level: $124,980

ARTHUR D. LITTLE, INC. (NO1-CP-55711)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays

Contractor's Project Director: Dr. Andrew Sivak

Project Officer (NCI): Dr. Virginia Dunkel

Objectives: To develop and standardize methods for performing *in vitro* transformation assays with BALB/c-3T3 cells and early passage Fischer rat embryo cells using chemical carcinogens as the transforming agents.

Major Findings: Task II-BALB/c-3T3 Cells - Populations of BALB/c-3T3 cells differ in their capacity in focus assays to undergo neoplastic transformation induced by chemical carcinogens. The sensitivity of various cloned populations to such transformation does not appear to depend on the saturation

density, the ability to metabolize polycyclic aromatic hydrocarbons or on the mode and distribution of chromosome number. Clone 1-13, derived from clone A31 by T. Kakunaga, (National Cancer Institute) is the most consistent in positive response to direct-acting carcinogens (n-methyl-N'-nitroso-N-nitroguanidine) and those requiring metabolism (3)-methylcholanthrene, benzo(a)pyrene) compared to the parent clone A31, clone 714 and sixteen clones isolated from the clone A31 population in the laboratory.

Task III - Fischer Rat Embryo Cells - Rat embryo cells did not exhibit a transformation response to chemical carcinogen treatment. Investigation of different gestation ages for culture initiation, different rat strains or cultures from single embryos, pooled single litters or pooled multiple litters did not result in a consistent demonstration of neoplastic transformation. Midway through the contract year, it was decided that the total effort should be concentrated on transforming BALB/c-3T3 cells.

Significance of Biomedical Research and the Program of the Institute: The carcinogenic risk to man from chemical agents now present in the environment or being introduced anew is largely unknown. The task of testing these materials by the classic chronic animal exposure protocols is formidable and prohibitive with respect to resources. While the short-term screening tests for mutagenicity with microorganisms are more reasonable in cost, they are at best only indicative of some genomic alterations and do not adequately reflect the process of carcinogenesis. The cell culture systems under study should provide standardized procedures to examine chemicals for carcinogenicity in short-term systems of modest cost that have genuine biological relevance to the process of carcinogenesis. Further, these cell culture systems should allow a more detailed explanation of the sequential cellular processes occurring in the development of neoplastic disease.

Proposed Course: Studies with the BALB/c-3T3 cells are underway (1) to examine the stability of clone 1-13 for chemically induced transformation, (2) to determine the optimum conditions for demonstration of transformation with respect to culture medium, serum, plating density, exposure time and assay time, (3) to determine conditions for use of microsomal activation systems, (4) to complete studies on known positive and negative carcinogens, and (5) to commence tests with blind, coded samples.

Date Contract Initiated: June 30, 1975

Current Annual Level: $218,969

BIOLABS, INC. (NO1-CP-45615)

Title: In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. Bruce C. Casto

Project Officer (NCI): Dr. Joseph A. DiPaola

Objectives: The specific objective of this contract is to study the process whereby chemical carcinogens promote viral oncogenesis and to determine if oncogenic and non-oncogenic viruses may similarily promote carcinogenesis by known chemical carcinogens.

Major Findings: This study has established that treatment of cells with chemical carcinogens and mutagens will increase the frequency of viral transformation in vitro. Approximately 150 chemicals including polycyclic hydrocarbons, aromatic amines, alkylating agents, inorganic metal salts, drugs, fuel or food additives, pesticides, herbicides, organic solvents and other industrial chemicals have been or are being tested in hamster cells for toxicity and their capacity or enhance viral transformation, induce DNA strand breaks, stimulate unscheduled DNA (repair) synthesis, induce mutations, and transform cells in vitro.

All of the chemicals tested that are known to be, or suspect, carcinogens in man or animals will enhance viral transformation with the exception of certain chemicals that may not be metabolized by hamster cells in vitro including; red dye #2, chloroform, trichloroethylene, N-nitrosodiethylamine, N-nitrosodimethylamine, urethane, thiourea, and ethylenethiourea. When incubated with a hamster liver S-9 activation system, N-nitrodimethylamine and urethane will enhance. Several chemicals such as manganous chloride and 2-chloro-1,3-butadiene, that do not cause enhancement when preincubated with confluent cells, are positive when added post-virus inoculation and cell transfer. Forty-two of the top 50 chemicals produced in billions of lbs/yr have been tested in the viral enhancement assay. Propylene oxide, vinyl acetate, and ethylene dichloride are positive for enhancement in hamster cells. In addition, inorganic metal salts have been tested with positive enhancement obtained with some of the metals.

Chemicals shown to enhance viral transformation may or may not cause DNA breaks and repair synthesis; however, all chemicals that break DNA or induce repair will enhance. Approximately 60 chemicals have been examined for their capacity to induce DNA strand breakage and of these, thirty-five cause DNA breaks. Of the 40 carcinogens tested for DNA repair in hamster embryo cells 40% are positive, whereas none of the 20 non-carcinogens are positive.

Enhancement of viral transformation by nickel sulfate and methyl methanesulfonate is associated with an increased integration of viral DNA into cell DNA as demonstrated by cRNA-DNA hybridization studies.

Chemicals that stimulate the frequency of viral transformation in Syrian hamster embryo cells are being tested in the same cell system for carcinogenic transformation without added virus. Nineteen carcinogens have been shown to transform these cells in a focus assay system. Cell lines derived from these foci have been characterized according to their: (1) tumorigenicity in animals; (2) capacity to clone in soft agar, concanavalin A, heparin, dextran sulfate, DEAE dextran or reduced serum; and (3) response to normal cell constituents. Those properties more closely associated with tumorigenicity were the ability to clone in soft agar and resistance to dextran sulfate and heparin.

Experimental models have been developed to study the interaction between viruses and chemical carcinogens in vivo. Tumors induced in hamsters by intradermal inoculation of 3-methylcholanthrene, followed by certain adeno-or herpes viruses at the same site, have shown decreased latent periods in contrast to tumors induced by 3-methylcholanthrene alone.

Significance to Biomedical Research and the Program of the Institute: In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled in vitro system. This would establish not only the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis, but also the role of chemicals in promoting viral carcinogenesis. Sensitive quantitative assays, may be used to detect potential carcinogenic or mutagenic chemicals in the environment.

Proposed Course: Diverse chemicals that are important to the human environment, including food additives, pesticides, herbicides, and other industrial chemicals will be examined for their ability to enhance viral transformation. The nature of the persistence of enhancement following chemical treatment will be the subject of future studies, especially the relationship between persistence of enhancement and the occurrence of DNA repair and breaks. Factors known to influence the toxicity and carcinogenicity of certain chemicals will be studied to determine their influence on the enhancement of quantitative transformation of cells by virus. Other types of cells and viruses will be employed to determine if the phenomenon of enhancement occurs in other systems. The influence of viruses on the frequency of transformation of hamster cells by chemicals will be explored using a quantitative, focus assay that does not require the use of feeder cells. Methods developed for chemical transformation of hamster cells will be used to investigate the sensitivity of other cell systems for in vitro transformation. Attempts will be made to determine the relationship between enhancement of viral transformation, DNA repair, DNA breaks, and chemical transformation of Syrian hamster cells.

Date Contract Initiated: June 9, 1971

Current Annual Level: $381,748

CHILDREN'S HOSPITAL OF AKRON (NO1-CP-55706)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays

Contractor's Project Director: Dr. Howard J. Igel

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: (1) Define aspects of carcinogen metabolism in adult skin epithelial cells of laboratory animals which relate to in vitro neoplastic transformation with chemicals. (2) Treat in vitro grown skin with suspected carcinogens and noncarcinogens and monitor for markers of neoplastic transformation in resultant cell populations. (3) Develop protocols for an in vitro bioassay of chemical carcinogens with emphasis on reproducibility, standardization, and sensitivity in the animal model of choice.

Major Findings: By direct measurement of benzo(a)pyrene metabolism in primary skin epithelial cells and fibroblasts from rabbit, hamster, mouse, guinea pig, and human skin cultures we have identified the rabbit and human epithelial cell as potential "metabolizer cells" for an in vitro transformation system. Normal human and outbred New Zealand White (NZW) rabbit skin epithelial were grown on a plastic substrate and treated with 12 chemicals (ultimate, pro and noncarcinogens) ranging in concentration from 0.1 to 200 ug/ml. Primary cultures were chosen for subculture based on the extent of epithelial cell toxicity. At various passage intervals, the population was monitored for morphological conversion, karyotype, liquid plating efficiency, soft agrose plating efficiency, and fibrinolytic activity. In human skin cells, no evidence of neoplastic transformation was detected up to 36 population doublings at which time control and treated cultures senesced. In rabbit skin cells treated with polycyclic hydrocarbons, morphological evidence of transformation was manifested beginning at 18 population doublings. The temporal acquisition, persistence of markers, and the chemical specific combination of phenotypic variants emerging on passage was correlated with the known carcinogenicity of each chemical. Solvent treated controls have yet to exhibit these markers up to 60 population doublings. High passage rabbit skin fibroblasts do not transform at doses of chemical which transform skin cells treated as primary cultures of high epithelial cell content.

Significance to Biomedical Research and the Program of the Institute: Chemically-induced in vitro transformation of diploid mammalian cells requires (1) Organ-specific procarcinogen metabolism, (2) the presence of sensitive and surviving target cells in the population, and (3) expression of the transformed phenotype. Defining the contribution of various cell types in organotypic primary cultures from epithelial organs such as skin will lead to in vitro bioassay strategies applicable to other epithelial organs. This knowledge could lead to relevant organ-specific bioassay of chemical agents causing epithelial cancer in man.

Proposed Course: Refine and define the rabbit skin transformation system by: (1) Screening rabbit genotypes to find inbred strains of sensitivity equivalent to NZW rabbits. (2) Reproduce entire system with several chemicals in that inbred strain. (3) Choose most predictive reliable phenotypic marker appearing after treatment with all chemical classes. (4) Determine tumorigenicity of rabbit skin cultures in autologous host and/or nude mice. (5) Examine the influence of chemical dose, epithelial cell content, promoters, and critical culture conditions, on the emergence of variant cells exhibiting a transformed phenotype.

Date Contract Initiated: June 30, 1975

Current Annual Level: $218,860

ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (YO1-CP-50200)

Title: NCI-ERDA Carcinogenesis Program

Contractor's Project Director: Dr. Theodore T. Odell, Jr.

Project Officer (NCI): Dr. Thomas B. Owen

Contract narrative is reported under the Interdisciplinary Programs and Resources Operational Unit.

ILLINOIS, UNIVERSITY OF (NO1-CP-23303)

Title: Temperature Sensitive Mutants in In Vitro Carcinogenesis

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. John Bader

Objectives: (1) Expose BHK 21 hamster cells to chemical carcinogens. (2) Analyze transformation by microscopic observation of morphological change and growth by soft agar under normally restrictive conditions such as sensitivity to temperature. (3) Manipulate clones of transformed cells for ability to grow under a variety of conditions in order to analyze the reasons for physiological changes responsible for malignancy. (4) Test transformed cells to establish the observed correlation between ability of plating in semi-solid medium and tumorigenesis in the hamster. (5) Test the feasibility of BHK cells in a quick, reliable carcinogen screening test.

Major Findings: Analysis of 4NQO and NMU-induced transformed BHK clones has revealed that those with temperature-restricted transformed phenotypes arise frequently over a wide range of doses. Careful studies of the induction, reversion and stability of these carcinogen-induced transformants indicate that carcinogens cause their malignant transformation by the induction of a somatic mutation.

Significance to Biomedical Research and the Program of the Institute: An exploitable system for the determination and analysis of chemical carcinogens is essential to progress in understanding carcinogenesis with the ultimate view of preventing and/or curing tumors induced by chemicals. Considerable progress has been made on developing such a system.

Proposed Course: (1) Adptation of BHK transformation system to a spot-test screening procedure suitable for large-scale carcinogen detection. (2) Application of the successful BHK experimental techniques to lines such as Wi-38 to establish the mutagenic basis of carcinogenesis in human tissue and possibly generate transformed clones where the transformed phenotype modulates with temperature. (3) Determination of the dominant recessive character of transformed phenotype in BHK cells and an analysis by complementation of the number of genes in BHK cell line which can cause transformation.

Date Contract Initiated: June 20, 1972

Current Annual Level: $180,223

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-55630)

Title: The Significance of Experimental Carcinogenesis Data to Man

Contractor's Project Directors: Dr. Helmut Bartsch
Dr. Ruggero Montesano

Project Officer (NCI): Dr. Virginia Dunkel

Objectives: The overall objectives of this program are the development of better criteria for the extrapolation of experimental carcinogenicity data to man and the validation and improvement of rapid screening tests which may have use in selecting chemicals for in-depth investigation and/or in predicting the carcinogenicity of environmental chemicals.

To pursue these objectives, the research focuses on four major topics: (1) comparative metabolism of carcinogens in human and animal systems; (2) studies on DNA repair processes; (3) mutagenicity tests in vitro; and (4) chemical carcinogenesis in vitro.

Major Findings: (1) To examine the nature of the carcinogen-activating enzymes in human liver, we have measured microsome-mediated mutagenicity in human biopsy livers and compared the results with those obtained with mouse or rat tissues. Mutagenicity assays were carried out in plate incorporation assays as described by Ames or according to a procedure adapted to test volatile compounds using histidine auxotrophic strains of S. typhimurium. Several biopsy specimens from human liver were active in converting various halogenated hydrocarbons and N-nitrosamines into mutagenic metabolites and this activity has been examined for a possible correlation with the aryl hydrocarbon hydroxylase activity present in these livers. (2) Recent studies in rats have shown that the persistence of certain alkylated bases in DNA, i.e., O^6-alkylguanine, may be a determining factor in the organ specific carcinogenicity of some N-nitroso compounds.

Using a single dose of dimethylnitrosamine (DMN), which produced a 30% incidence of liver tumors in hamsters, we have observed a persistence of O^6-methylguanine(O^6-meGUa) in the DNA of this organ, whereas the same alkylated base is rapidly excised from liver DNA of rat, an organ that does not develop tumors after a single administration of DMN. In another series of experiments, in order to explain the organ specificity of DMN under different dose schedules, we have explored the possibility that inhibition of the O^6-meGua excision system plays an important role in the development of liver tumors in rats following chronic administration of DMN.

Rats were exposed chronically to unlabelled DMN (25 ppm in the drinking water) then given a single dose of N-($_3$H)-methyl-N-nitrosourea (MNU) (10 mg/kg). This compound, and not DMN, was chosen, since MNU does not need to be metabolized in order to react with nucleic acids; thus, excision repair

capacity in control and DMN-pretreated rats could be determined under conditions in which the initial extent and the time of maximum DNA alkylation were identical. The results as expressed by the O^6-meGua:7-meGua and 3-meA:7-meGua ratios are almost identical in control and DMN-pretreated animals.

The possibility that O^6-meGua might specifically accumulate in target organ DNA during chronic administration of DMN was also investigated. In BD rats such a regime induces tumors of the liver but not of the kidney or lung. BD-IV rats were treated with ^{14}C-DMN (2 mg/kg/day) by gavage on weekdays and the rats were sacrificed at 2, 4, 8, 16, and 24 weeks after the beginning of the treatment and in each case 72 hours after the last DMN administration. The amounts of 7-meGua and O^6-meGua in liver, lung and kidney DNA were then determined. Although a large amount of 7-meGua was present in liver DNA, no O^6-meGua was detected throughout the period of the experiment, indicating that, at least under present experimental conditions, chronic treatment with DMN does not lead to a specific accumulation of O^6-meGua in target organ DNA. However, O^6-meGua did accumulate in the DNA of kidney and lung, organs in which tumors are not induced during chronic treatment with DMN.

(3) Various parameters which contribute to the efficiency and/or reproducibility of the S. typhimurium microsome mutagenicity test have been examined. A quantitative comparison was made of the mutagenic effects of a series of aliphatic N-nitrosamines in a liquid incubation system and in the plate incorporation assay in the presence of a rat liver fraction using the strain TA1530. The absolute and relative mutagenic activities observed in the two systems were not identical, the N-nitrosamines with less than two carbons on the side chains being more active in the liquid system and the other N-nitrosamines in the plate incorporation assay. To explain these differences in the mutagenic effect, it was demonstrated that the plate incorporation assay in soft agar is more effective in detecting chemicals whose metabolic conversion into mutagens occurs at a low rate.

The plate incorporation assay with S. typhimurium strains was also adapted to test volatile compounds by exposing Petri dishes containing bacteria and fortified post-mitochondrial supernatants to various gaseous mixtures (vinyl chloride, vinylidene chloride, 2-chlorobutadiene, etc.) of the test compound in air.

In addition, a microsome-mediated mutagenesis has been established with Chinese hamster V79 cells. With this system, various carcinogenic and non-carcinogenic N-nitrosamines as well as the influence of inducers and inhibitors of mixed-function oxidase have been investigated. With the exception of N-nitrosomethylphenylamine, all carcinogenic nitrosamines were mutagenic in the V79 Chinese hamster cells, but only in the presence of the S-15 fraction. The two non-carcinogenic compounds, N-nitrosodiphenylamine and N-nitrosomethyl-tert-butylamine, had a mutagenic effect.

(4) Studies on the transformation of epithelial-like cells from the liver of BD rats, 10 days or 8-10 weeks of age, have shown that these cells can be transformed in vitro as indicated by the development of carcinomas following

back-transplantation into syngeneic rats. In most of the cultures examined, a spontaneous transformation occurred in cells maintained in culture approximately 50 weeks. However, treatment with some carcinogens (DMN and MNNG) resulted in transformation of the cells at an earlier stage.

A microsome-mediated transformation system is being established using C3H 10T 1/2 mouse cells as an extension of microsome-mediated mutagenesis of V79 cells. Various chemical carcinogens which require metabolic activation are under test in this system.

Significance to Biomedical Research and the Program of the Institute: These studies are designed to develop and evaluate various test systems for the identification of environmental carcinogens as well as to provide criteria for a better assessment of the significance of experimental data to man.

Proposed Course: The activity will continue along the same lines as indicated in the Major Findings section.

Date Contract Initiated: September 1, 1974

Current Annual Level: $190,717

INVERESK RESEARCH INTERNATIONAL (NO1-CP-75858)

Title: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

Contractor's Project Director: Dr. Douglas B. McGregor

Project Officer (NCI): Dr. Virginia Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: The objective of this project is to validate certain microbial mutagenicity tests for their predictive value for carcinogenic potential. Approximately 80 chemicals supplied by NCI are being tested for mutagenicity in a double-blind study using five S. typhimurium strains and three E. coli strains.

Major Findings: Substance 25860 has been found mutagenic in S. thypimurium TA 1535, TA 100, TA 1538 and (weakly) TA 98 and in E. coli WP2 uvrA$^-$.

Proposed Course: Coded compounds will be tested at approximately weekly intervals until approximately 80 compounds have been tested.

Date Contract Initiated: December 29, 1976.

Current Annual Level: $129,849

JOHN L. SMITH MEMORIAL FOR CANCER RESEARCH, PFIZER INC (NO1-CP-55703)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays-Task I

Contractor's Project Director: Dr. John S. Wolff, III

Project Officer (NCI): Dr. Virginia C. Dunker

Objectives: Develop methodology, using normal or Rauscher-leukemia virus (RLV)-infected rat embryo cells, suitable for in vitro assessment of the carcinogenic potential of chemicals.

Major Findings: Two different culture strains of Fischer rat embryo cells have been established and infected with RLV. Both virus-infected cell strains are susceptible to transformation by carcinogenic chemicals, including 3-methylcholanthrene, dimethylbenzanthracene, dimethylnitrosamine, acetylaminoflourene, 4-nitroquinoline-N-oxide, benzo(a)pyrene, dibenzanthracene and urethane, but are unaffected by such noncarcinogenic compounds as diphenylnitrosamine, phenanthrene and benzo(e)pyrene. The transformation phenomenon is observed as loss of contact inhibition, resulting in focus formation, in the treated cells. The focus-forming cells grow without anchorage dependence and form tumors in appropriate animals, confirming their transformed state. Both cell strains are about equally sensitive to transformation by the variety of chemicals tested, and have similar growth characteristics. In addition, while transformation-insensitive clones have been obtained, no clone has proven more sensitive than the parent to transformation by the range of compounds tested. Assay validation is now in progress, with one of the cell strains being tested for susceptibility to transformation by 90 coded samples of known carcinogenic activity in animals.

While virus-infected cells are more sensitive than uninfected counterparts the normal cells do, under certain circumstances, respond to carcinogen treatment. Studies are underway, using several newly-established rat embryo cultures, to determine the culture conditions required for use of these noninfected cells in a reproducible carcinogen assay.

Significance to Biomedical Research and the Program of the Institute: Each year, many new chemicals are synthesized for use in consumer products or as intermediates in other processes. Some unknown number of these is likely to be carcinogenic. Present bioassay protocols are too expensive and time-consuming to permit carcinogenicity assay of each of these new compounds. It is expected that a prescreen, combining in vitro carcinogenicity assay with other procedures, will identify compound candidates for bioassay. The RLV-infected rat embryo cells are one system which is sensitive to transformation by some known carcinogens. Development of in vitro carcinogenesis protocols using this model should provide one means of bioassay prescreen.

Proposed Course: (1) Continue assay validation with RLV-infected rat embryo cells with 90 coded compounds. (2) Continue investigation of growth characteristics of RLV-infected cells, with the objective of developing a more rapid, more sensitive assay. (3) Determine utility of non-virus-infected rat cells for carcinogen assay.

Date Contract Initiated: June 23, 1975

Current Annual Level: $139,023

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CP-65804)

Title: Production of an In-Bred Syrian Hamster Colony

Contractor's Project Director: Ms. Miriam R. Sacksteder

Project Officer (NCI): Mr. Clarence Reeder

Objectives: The increasing importance of screening methodology for the detection of environmental carcinogens of significance to man requires the use of animals and/or their tissues of defined parentage and raised under optimum conditions. Cultures of the embryonic cells of the Syrian hamster have been found to undergo malignant transformation when exposed to relatively low concentrations of recognized carcinogens in vitro. To expand the application of these techniques requires a continuing supply of timed pregnant rodents.

The objective of this project is to maintain, breed and monitor a colony of approximately 550 Graffi strain Syrian hamsters from which a minimum of six timed pregnant females per week are furnished to the NCI and its associated contractors. These pregnant females are distributed as directed by the Project Officer.

Excess weanling hamsters as well as retired breeders are available to other institutions as designated by the Project Officer.

The colony is maintained in isolated quarters without contact with other animals. Clear plastic cages (as opposed to opaque cages) are used to facilitate successful breeding. The colony is routinely monitored for its microbiological flora and continues to be free of enteric pathogens.

During the contract period (9/1/76-3/31/77) we have shipped 108 pregnant hamsters, 30 weanling and 10 retired breeders. We have reached the colony size as specified in the contract. However, this number fluctuates between 500-550 animals.

We shall continue production and distribution at the present level unless advised to the contrary.

Date Contract Initiated: September 1, 1976

Current Annual Level: $31,465

LITTON BIONETICS, INC. (NO1-CP-65853)

Title: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogen Screening

Contractor's Project Director: Dr. Dale W. Matheson

Project Officer (NCI): Dr. Virginia C. Dunkel
(FDA): Mr. Kenneth Palmer

Objectives: To develop and validate the $TK^{+/-}$ mutant L5178y mouse lymphoma cell system as an assay system for detecting chemical mutagens. The developmental stage includes rigorously defining test conditions including evaluation of rodent liver metabolic activation systems; the validation component includes testing 100 chemicals under blind condition. Ultimately these data will be compared with those from carcinogenesis studies on these same chemicals and used to evaluate the relationship between mutagenesis and carcinogenesis and the predictive value of the mouse lymphoma assay for carcinogenesis.

Major Findings: The major finding anticipated, that of using the test as a predictor of chemical carcinogenesis, cannot be determined until the end of the second year when all the correlative data has been evaluated.

Significance to Biomedical Research and the Program of the Institute: If this short term assay using mammalian cells can be used to detect mammalian and especially human carcinogens, then it has potential value to man and his environment, and could have a significant effect on carcinogenesis testing.

Proposed Course: To continue the development of the mouse lymphoma assay using mammalian liver metabolic activation systems and known mutagens and to validate the assay under blind conditions using known carcinogens and non-carcinogenic analogues and chemicals currently under test bioassay program.

Date Contract Initiated: September 30, 1976

Current Annual Level: $179,053

LITTON BIONETICS, INC. (NO1-CP-65856)

Title: Validation and Utilization of Microbial Mutagenesis Systems as a Prescreen for Chemical Carcinogens

Contractor's Project Director: Dr. David J. Brusick

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: Conduct double blind studies on approximately forty (40) coded chemicals in a microbial mutagenesis bioassay using Salmonella typhimurium and Escherichia coli. The results are to be used in evaluating the correlative relationship between mutagenesis and carcinogenesis.

Major Findings: No major findings anticipated since all chemicals tested are coded and will not be identified until all tests have been completed.

Significance to Biomedical Research and the Program of the Institute: If a series of short-term submammalian test systems are found to be highly accurate in predicting mammalian carcinogens, the overall nature of the carcinogenesis program of NCI might well be modified significantly.

Proposed Course: Conduct double blind mutagenesis studies on a series of chemicals and compare the results to the results of lifetime carcinogenesis bioassays in rodents using the same agents.

Date Contract Initiated: September 30, 1976

Current Annual Level: $159,840

MICROBIOLOGICAL ASSOCIATES (NO1-CP-55670)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assay

Contractor's Project Director: Dr. Nirmal K. Mishra

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: The objective of the project is to evaluate and determine the usefulness and reliability of in vitro cell transformation systems as initial assays in determining the carcinogenic potential of chemical compounds. Experimental condition for cell growth and the frequency and type of transformation obtained have been described in the literature for a number of systems. Among the systems to be considered are those in which direct action of a carcinogen on the cell leads to transformation and those in which the activity of a compound is mediated by appropriate metabolic activation, either in vivo or in vitro, or by its ability to enhance transformation of virus infected cultures.

Major Findings: A multicomponent test system based on Fischer rat embryo (FRE) cells preinfected with RLV and using rat liver homogenate is being standardized as an assay system for the detection of chemical carcinogens. The endpoints being measured are gene mutation (ouabain resistance) and cellular transformation. Eight known carcinogens and five structurally related non-carcinogenic analogues were tested in this system for standardization purposes. Except for one carcinogen, N-2-fluoronylacetamide, all

chemicals exhibited transforming activity. Furthermore, all transforming agents produced stable ouabain resistant cellular subpopulations in this system.

Using morphological transformation as the single endpoint, a variety of chemicals are being tested in blind studies. Assays on fifteen coded chemicals have been completed and 12 others are now under test. Since there is the need for exogenous metabolic activation in this system attempts are presently being made to standardize various induced and uninduced liver homogenates in terms of specific component of the total enzymatic activity such as aryl hydrocarbon hydroxylase.

Some basic inquiries regarding the role of leukemia virus in the promotion of transformation and mutagenic activity in the test system have been made and have yielded surprising results. It was found that while RLV infection profoundly affected the rate of transformation in FRE cells by chemical carcinogens, neither basal nor chemically induced gene mutation rates were modified by virus infection.

Proposed Course: (1) To continue to test (double-blind) various groups of procarcinogens, ultimate carcinogens and noncarcinogens in order to establish a reliability index of the assay; (2) to improve the sensitivity of the assay by determining the conditions required for metabolism of various procarcinogens; (3) To improve the reliability of the assay system with known chemicals.

Date Contract Initiated: June 27, 1975

Current Annual Level: $197,992

NCI-FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CO-25423)

Project #10

Title: In Vitro Carcinogenesis

Contractor's Project Manager: Dr. Roman Pienta

Project Monitor (NCI): Dr. Virginia C. Dunkel

Contract narrative is reported under Interdisciplinary Programs and Resources Operational Unit.

NEW YORK MEDICAL COLLEGE (NO1-CP-65855)

Title: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

Contractor's Project Director: Dr. Herbert S. Rosenkranz

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: (1) To develop short-term microbial assay procedures useful in predicting the carcinogenic potential of chemicals (2) To evaluate the reliability of existing microbial systems in achieving this purpose.

Major Findings: (1) Samples are submitted in a coded fashion. To date 15 coded test agents have been received. Tests on these are at various stages of completion. (2) It was demonstrated that the standard Salmonella mutagenicity assay can be adapted to activation of procarcinogens by cell-free extracts from anaerobic bacteria. This will permit an evaluation of the role of the anaerobic flora in the etiology of colon cancer.

Significance to Biomedical Research and the Program of the Institute: Identification of cancer-causing agents in the environment is a prerequisite for the elimination of such agents from our surroundings. The present study seeks to develop and evaluate rapid, simple and inexpensive, microbial procedures to be used as preliminary screens for chemical carcinogens.

Proposed Course: (1) To complete the requirements of this project, i.e. to determine the mutagenicity and DNA-modifying activity of test-agents as they are received. (2) To further refine the existing microbial assay procedures to increase their reliability as prescreens for cancer-causing agents.

Date Contract Initiated: September 30, 1976

Current Annual Level: $94,647

NORTH CAROLINA, UNIVERSITY OF (NO1-CP-55707)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays

Contractor's Project Director: Dr. Joe W. Grisham

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: The objectives of this contract are to develop methods and procedures for detecting chemically-induced transformation of cultured liver epithelial cells and to use this system to screen 90 reference chemicals (both carcinogens and their noncarcinogen analogs) supplied by the National Cancer Institute.

Major Findings: Several lines of epithelial cells have been established from rats of the inbred Fischer-344 strain and clones have been isolated. Epithelial cells from liver grow as a one-cell thick layer and adjacent cells are joined by attachment complexes. Cellular surfaces are densely covered with microvilli and some nontransformed lines produce plasminogen activators. The doubling time of all lines is near twenty-four hours. Proliferation of nontransformed cells is density inhibited and is suppressed in soft agar.

Different cell lines vary in their ability to metabolize benzo(a)pyrene to diols and this metabolic activity is influenced by the type of medium. Despite different capabilities to metabolize benzo(a)pyrene, all cell lines studied are relatively resistant to the toxic effects of this chemical; exposure for 24 hours to 5ug benzo(a)pyrene/ml of culture medium reduces the colony forming efficiency of liver epithelial cells by less than 50%. In contrast, N-methyl-N'-nitroso-N-nitroguanidine, which does not require metabolic activation, is highly toxic for all lines of liver epithelial cells; the colony forming efficiency is reduced by 50% following exposure of liver epithelial cells for 90 minutes to concentrations of MNNG that range between 0.15 to 0.60 ug/ml.

Recent studies with fibroblastic cells indicate that high concentrations of serum inhibit expression of cellular transformation. All clones of liver epithelial cells grow optimally at serum concentrations of 2.5% to 5% (range examined 2.5% to 25%) in Richter's Improved Minimal Essential Medium. High rates of spontaneous transformation, as indicated by chromosomal abnormalities and ability to grow in soft agar, have characterized cells cultured continuously at low serum concentrations. In contrast, cells cultured at high serum concentrations appear resistant to either spontaneous or induced transformation.

Proposed Course: (1) Evaluate the ability of cultured liver epithelial cells to metabolically activate other procarcinogens. (2) Determine the toxicity of various carcinogenic and noncarcinogenic chemicals for liver epithelial cells, by determining their fractional survival (colony forming efficiency) after exposure to graded concentrations of chemicals. (3) Seek to identify specific, carcinogen-induced transformation of liver epithelial cells following their exposure to selected chemicals, and determine the temporal pattern of the cells' structural and behavioral changes. (4) Evaluate further the relationship between serum concentrations and spontaneous and induced neoplastic transformation. (5) Continue to establish and test other clones of liver epithelial cells in order to attempt to identify a clone with optimal properties for transforming in response to both ultimate and procarcinogens. (6) If and when system is reproducible, screen unknown chemicals supplied by NCI.

Date Contract Initiated: June 30, 1976

Current Annual Level: $95,813

OHIO STATE UNIVERSITY (NO1-CP-43276)

Title: In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. George Milo

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: To determine whether known direct acting chemical carcinogens will induce morphologic and neoplastic transformation of selected human cells in vitro. To determine whether hormones act as potentiators of the transformation process in the presence of the chemical carcinogens. To determine whether direct acting carcinogens interact with feline sarcoma virus to enhance virus transformation in human cells, and to examine the interaction of carcinogens and hormones in virus transformation of human cells in vitro.

Major Findings: Alterations of growth characteristics, of human embryonic lung cells, spleen, liver, prostrate, intestine from males and human foreskin cells were obtained following treatment with benzo(a)pyrene (BP), 4-nitroquinoline-1-oxide (4NQO), N-methyl-N-nitrosoguanidine (MNNG), N-acetoxy-N-2-fluorenylacetamide (N-Ac-AAF), Aflatoxin B_1, Propane Sultone, ultraviolet 254 nm and γ-rays. Cells that were treated sequentially, singularly or continuously with each of the carcinogens inhibited transitory altered profiles, i.e. altered morphology, extension of lifespan and growth in soft agar. Treatment of the cells with 7,12-DMBA stimulated scheduled DNA synthesis, whereas 3-methylcholanthrene (3-MCA) elicited no cellular or molecular responses. The direct acting carcinogens propane sultone, N-Ac-AAF, 4NQO, MNNG, ultraviolet and γ-rays induced unscheduled DNA systhensis. Treatment with the direct acting carcinogens and 17-β estradiol, diethylstilberstrol, cortisone-Ac, and hydrocortisone promoted scheduled DNA synthesis but inhibited unscheduled DNA synthesis after DNA damage. When cells in the S phase were treated with carcinogens, they showed permanent alteration of cellular and culture characteristics. At the present, 20 different lines have been developed from the treated cells. The transformed cells grow at 41°C in the absence of CO_2 form colonies in 0.3% agar and 1.0% Methocel and will grow in growth medium supplemented with 1% FBS. Cells reach a confluent density in 50% of the time required for low passage cells confluency. Mix-function oxygenase activity in low passage human lung and skin cells was induced by BP or DMBA but not 3-MCA and hydroxylated products were formed. The BP product induced damage to cellular DNA, whereas no damage was observed with 7,12-DMBA and 3-MCA. Non-carcinogenic chemicals and weak carcinogens were examined in the cell systems and found to act in a similar manner, that is, they induced scheduled DNA synthesis, but no DNA damage and did not activate the mix-function oxygenase enzymes.

Only these carcinogens which induced DNA damage enhanced SV40 virus transformation. BP, 4NQO and MNNG either enhanced or inhibited Synder-Theilen Feline Sarcoma Virus transformation. With both the oncogenic DNA and RNA virus transformation assays, addition of chemical carcinogens altered virus transformation.

Significance to Biomedical Research and the Program of the Institute: Although non-human mammalian cells have been transformed in vitro with known chemical carcinogens, there is no report of transformation of normal human cells by chemical carcinogens. It is of major importance to determine the conditions necessary to achieve neoplastic transformation of human cells in vitro by chemical carcinogens. An understanding of the process by which these carcinogens at least initiate the transformation process can be obtained by analyzing their ability to promote viral transformation by human cells.

Proposed Course: To continue treating human foreskin cells in vitro with other carcinogens to study the changes associated with the transformation process. Different systems will be examined in order to aid in the detection and quantitation of the transformation events in normal human cells. The interaction between steroids and direct acting carcinogens will be studied to determine role of each in the transformation process. FSV will be used to evaluate the interaction between either carcinogens or hormones with the virus and the human foreskin cells.

Date Contract Initiated: March 4, 1974

Current Annual Level: $154,939

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP-65831)

Title: Carcinogenesis In Vitro; Initiation and Promotion

Contractor's Project Director: Dr. Charles Heidelberger

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To develop studies with the 10T 1/2 cell line to bring it to the point where it would be feasible to evaluate its potential as a pre-screen for environmental carcinogens. The basic goals of the project are: (1) developing a liver mircosomal-activating system; (2) improving and quantitating methods of scoring for transformation by the uptake of tritiated thymidine following confluence, the use of soft agar, plasminogen activation and microdetection of embryonic antigens; (3) developing an initiation-promotion system as a potential pre-screen for carcinogens; and (4) developing methods for the assay of syn-carcinogenesis as a potential pre-screen for combination of weak carcinogens.

Major Findings: C3H/10T 1/2 cells are transformable by 3-methylcholanthrene (MCA), benzo(a)pyrene (BP), and 7,12-dimethylbenz(a)anthracene (BMBA) at .025 ug/ml. When the cells were treated with 0.1 ug/ml of the above carcinogens, there was no or very little transformation; this was a subeffective concentration.

The tumor promoting phorbol ester, 12-O-tetradecanoyl phorbol-13 acetate (TPA), was found to be nontoxic and nontransforming to these cells at a concentration of 0.1 ug/ml. However, when the cells received 0.1 ug/ml of TPA 5 days after 0.1 ug/ml of the above hydrocarbons, the frequency of transformation was significant. By contrast, cultures that received TPA immediately after the treatment with three hydrocarbons in transforming (0.25 ug/ml) concentrations the frequency of transformation was diminished. It was found that the above amount of TPA inhibited cell proliferation for 48 hours. Thus, it appeared that the transformation of those cells that were initiated by hydrocarbons could be promoted by TPA treatment provided that the initiated cells were allowed to undergo divisions to allow "fixation" of the transformed state. In addition to TPA, other phorbol esters e.g. PDD and 4-α-PDD and phorbol itself were examined for their promoting activity. PDD and 4-α-PDD were found to be weak promoters and phorbol was inactive. When TPA treatment preceded MCA treatment no enhancement of transformation was observed.

In addition to chemical carcinogens, one physical carcinogen, UV light, was found to initiate the cells in the process of carcinogenesis. There have been no reports that UV light can transform cultured mouse cells, although it is considered to be highly carcinogenic and produces skin cancer in rats, mice, and humans. The C3H/10T½ cells were irradiated once with 10,25,50, 100, 150 and 200 ergs/mm^2 from a UV light. No transformation was observed at any of the above doses after six weeks of culture, and no cytotoxicity at the lowest two doses. However, when the irradiated cells were cultured in a medium containing 0.1 ug/ml of TPA starting from 0 to 120 hours after the irradiation, a high frequency of transformation was produced in all cases. When the cells were initiated with subeffective concentrations of methylcholanthrene (0.1 ug/ml) followed by UV irradiation at different intervals, no transformation occurred. Moreover, different doses of UV irradiation followed by 0.1 ug/ml of MCA or multiple applications of UV radiation of different doses at different intervals did not yield transformation (unpublished data). Thus, it is evident that UV light acts as a pure initiator in our system and a promoting agent is required for the completion of the carcinogenic process. When the cells were treated first with TPA, the promoter, and then with UV light, there was little or no transformation. It was also found that a stimulus to cell proliferation after initiation was not sufficient to promote the transformation, although it was necessary. Therefore, TPA does something in addition to stimulation of cell division.

At present, a series of noncarcinogenic or weakly carcinogenic compounds are being tested in this system with promoters to determine whether they act as initiators. If so, this should aid in the screening of weak carcinogens in the environment.

The molecular events taking place after initiation and promotion were considered to be worth investigating. At first, DNA synthesis was measured both by autoradiography and by the uptake of tritiated thymidine in DNA from the cells treated with MCA, UV light, or different active and inactive promoters i.e., different isomers of TPA. It was found that active promoters had an inhibitory action on DNA synthesis for about 12 hours after application, and then there was a compensatory increase; the inactive promoters had no such effect.

It has also been found with C3H/10T 1/2 cells that nontransforming doses of the same or different polycyclic hydrocarbons can be added with proper timing and produce significantly increased transformation. This syn-carcinogenesis was not marked when the cells were first treated with a nontransforming concentration of MCA (0.1 ug/ml) and 1-3 days later with another nontransforming concentration of MCA, BP or DMBA. When the cells were treated first with a non transforming concentration of BP or DMBA and then with the same concentration of the other compound at different intervals, the effect was less marked.

The ability of cells to grow in semi-solid medium is currently being studied using agarose as a suspending agent; agar exerted cytotoxicity upon chemically transformed cells. More than 20 chemically transformed cell lines have been examined. All those that are oncogenic upon inoculation into suitable host animals are capable of growing in agarose. Some cell lines have been isolated which grow in agarose but are not oncogenic. The ability to grow in agarose frequently appears to exhibit a dependence upon the passage number of cells. Agarose plating efficiencies are low shortly after chemical transformation and increase as the cells are passaged. The C3H/10T 1/2 parent line shows no capacity to grow in agarose regardless of passage number. Current investigations are centering on the nature of the agar toxicity effect, the increase of the growth capacity in agarose with passaging, and the nature of cells which grow in agarose but are not oncogenic.

In a series of experiments it was seen that C3H/10T 1/2 cells with added fortified liver microsomes could metabolize aflatoxin B_1 and dimethyl-nitrosamine, as evidenced by cytotoxicity. The 9000 x g supernatant of homogenized rat liver was taken as the microsomal preparation. This with added co-factors (NADP, NADPH, G-6P, and NADH) was mixed with PBS (HEPES) containing the carcinogens, which was then added quickly to the culture. The whole treatment time was seven hours. Transformation experiments with these compounds, which are not metabolized by C3H/10T 1/2 cells are now being carried out.

Significance to Biomedical Research and the Program of the Institute:
The major goal of this project is to develop an assay for carcinogenesis screening with 10T½ cell culture. A rapid and convenient system to screen different environmental chemicals for their carcinogenic potential is extremely necessary today. Screening the combination of subthreshold doses of different carcinogens and initiators and promoters is also

essential for environmental surveillance, since in the environment many carcinogens are present at subthreshold concentrations and may contain substances which act as promoters.

In addition, we are investigating the molecular mechanisms of promotion, with particular attention to DNA, RNA, and protein synthesis, induction of ornithine decarboxylase, and induction of protease activities.

C3H/10T ½ cells are easily transformable by polycyclic hydrocarbons, virus, x-ray and UV radiation. Thus, the system has the potentiality to be used to screen environmental carcinogens and promoters. The main drawback in this system is that these cells cannot metabolize the compounds like dimethylnitrosamine, aflatoxin B_1, AAF, etc. Preliminary results show that this difficulty can be overcome by adding a liver microsomal system.

Proposed Course: We propose: (1) To continue these experiments until a rapid and convenient system to screen different environmental chemicals for their carcinogenic action is developed by (a) developing a liver microsomal activating system with 10T ½ cells; (b) improve the methods of scoring for transformation by the use of soft agarose. (2) To develop a system for the assay of syncarcinogenesis as a potential prescreen for combination of weak carcinogens. (4) To study the effects of promoters on the biosynthesis of DNA, RNA, and proteins in these cells.

Date Contract Initiated: September 1, 1976

Current Annual Level: $64,571

STANFORD RESEARCH INSTITUTE (NO1-CP-55701)

Title: Development of Detailed Methods and Protocols for Carcinogenic Screening Using Cell Culture Assays

Contractor's Project Directors: Dr. Gustave Freeman
Dr. Douglas Fodge

Project Officer (NCI): Dr. Virginia C. Dunkel

Objective: To evaluate and determine the usefulness and reliability of a host-mediated in vivo-in vitro system as an initial assay for the oncogenic potential of chemicals.

Major Findings: To establish the usefulness of an in vivo-in vitro cell culture system for identifying oncogenic substances, a systematic protocol has been developed to ensure that comparable amounts of the chemicals are injected into pregnant hamsters. Guidelines for determining these doses are based on the acute toxic properties of each chemical. Because few such studies have been performed on Syrian hamsters, a dose-response curve was

established first for each chemical by injecting it into adult animals. After the lowest lethal dose was determined, decreasing amounts of each chemical (LD_{LO} x 0.75, x 0.35, x 0.1 and x 0) were injected into 10-day timed-pregnant hamsters for determination of the fetal dose response $FeLD_{50}$). The dams and surviving offspring have been maintained throughout their natural lifetime to be observed for tumor development. To date, significant numbers of tumors have developed in dams exposed to three of the carcinogens, whereas only a few tumors have developed in dams exposed to noncarcinogens or in control dams. None of the offspring of any dams have developed tumors yet.

Nine major experiments to examine in vivo-in vitro transformation were conducted during the year. In these experiments, the $FeLD_{50}$ x 0.8 for individual chemicals was injected into pregnant hamsters, and the embryos were removed after various periods of gestation and placed in cell cultures.

A battery of tests to distinguish normal from transformed cells was used. Several tests, derived from studies for virus-transformed chick and hamster embryo cells, have been examined for their usefulness in embryonic hamster cells' cultures. These include growth rates, cloning efficiency, cytology, and tumor induction in syngeneic hosts. Preliminary data indicate that only quantitative differences exist between hamster cell cultures and the other species of embryonic cells relative to these parameters.

No evidence of transformation resulted from exposure to carcinogenic chemicals or to their analogs in experiments 1 through 4. These results prompted us to investigate the making of variations in the cultures medium that might enhance the prospects of identification of transformation. In experiments 5 through 9, the serum content and pH of the culture medium were varied. In experiments 5, 6, 8, and 9, embryonic hamster cell cultures exposed transplacentally to DMBA grew as if they were transformed. However, solvent-treated cultures in experiments 8 and 9 have behaved similarly. Thus, the in vivo-in vitro cell culture system for studying chemical carcinogenesis has shown interesting but unpredictable results in about 50% of our recent experiments. We are attempting to explain why earlier experiments gave unpredictable results and also why some untreated control cultures appear to be transformed.

Significance to Biomedical Research and the Program of the Institute: The host-mediated in vivo-in vitro cell culture system should be useful for scanning the large number of chemicals that may affect humans oncogenically. Moreover, the system may be important for identifying the mechanisms of tumor induction by such chemicals because it ensures the metabolic conversion of precursor or "incomplete" chemicals into oncogenic forms and also utilizes the speed and relative reliability of the in vitro assay. Thus, methods to develop activation of indirectacting chemicals in this whole-animal system should be advanced by this means. Moreover, these investigations will provide new information about the nature of transplacental oncogenesis, a subject of growing importance and intensive discussion.

Proposed Course: Continue developing the methodology of the host-mediated in vivo-in vitro cell culture bioassay system with new experiments.

Reduce the variability in the host-mediated system by using a selected responsive inbred line of Syrian hamsters.

Investigate the optimum sensitivity of the bioassay by varying the schedules for both the chemical injection and embryo delivery during gestation.

Investigate the influence of sex and age of host animals (after injection of transformed cells) on the sensitivity and reliability of the in vivo-in vitro test.

Investigate the probability that oncogen-sensitive tissues and cells other than fibroblasts can be discovered in the hamster by allowing offspring of oncogen-treated mothers to survive indefinitely. Should tumors develop, cell cultures will be prepared from fresh embryonic organs of such affected tissues for transformation studies.

Date Contract Initiated: June 29, 1975

Current Annual Level: $140,575

STANFORD RESEARCH INSTITUTE (NO1-CP-65854)

Title: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening

Contractor's Project Director: Dr. Ann D. Mitchell

Project Officers (NCI): Dr. Virginia C. Dunkel
(FDA): Mr. Kenneth Palmer

Objective: To evaluate and determine the usefulness and reliability of an in vitro forward mutagenesis assay system using L5178Y mouse lymphoma cells for the prescreening of potential chemical carcinogens.

Major Findings: None thus far; the contract has been underway for only a short period of time.

Significance to Biomedical Research and the Program of the Institute: Since the number of potentially hazardous chemicals that may significantly affect human population groups or the general population far exceeds the capacity of existing long-term animal and carcinogenesis test systems, there is a need for rapid short-term tests such as the mouse lymphoma mutagenesis assay system that may be used in a matrix for the initial evaluation of chemicals for possible carcinogenic potential.

Proposed Course: Initial phases concern (1) testing compounds of known chemical carcinogenicity in order to further characterize and increase the efficiency of the assay system and (2) developing and evaluating an appropriate in vitro metabolic activation methodology for use with the mutagenesis assay system. When there is a sufficient confidence with the system, approximately 100 chemicals consisting of both carcinogens and non-carcinogenic analogues will be tested double blind to determine and validate the response of the system.

Date Contract Initiated: September 30, 1976

Current Annual Level: $194,042

STANFORD RESEARCH INSTITUTE (NO1-CP-65857)

Title: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

Contractor's Project Director: Dr. Vincent F. Simmon

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: Validation of microbiological mutagenicity assays as pre-screens for chemical carcinogens using chemicals not identified to the experimenter (blind assays). Determination of best species for preparation of liver homogenate metabolic activation system (mouse, hamster, or rat). Determination if Arochlor 1254 (a polychlorinated biphenyl) pretreatment of the animals increases the probability of detecting carcinogens as mutagens. Determination of the reproducibility of the assay procedure (4 contract labs are conducting identical assays).

Major Findings: Since the compounds are coded, it is difficult to assess our findings. However, of 11 compounds tested to date, those in which mutagenicity was stimulated by or required metabolic activation, Arochlor pretreatment significantly increased the mutagenicity. One chemical was mutagenic only with the activation system from Arochlor pretreated animals and one compound was mutagenic with mouse and hamster liver preparation, but was not mutagenic with the rat liver preparation.

Significance to Biomedical Research and the Program of the Institute: Since the majority of cancers appear to be caused by chemical or physical agents, rapid, reliable assays for detecting chemical carcinogens are needed. The results from this contract (and three similar contracts) should enable an accurate evaluation of the predictive accuracy of microbial mutagenicity assays.

Proposed Course: Continue testing compounds as received until the contract is fulfilled (80 compounds).

Date Contract Initiated: September 30, 1976

Current Annual Level: $85,726

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-43257)

Title: Carcinogenesis Bioassay Data System (CBDS) Operational Support

Contractor's Project Director: Dr. Kantilal Patel

Project Officer (NCI): Dr. Kenneth Chu

Objectives: This project links data and information among the bioassay program contractors, the Bioassay Operations Segment, the Carcinogen Bioassay and Program Resources Branch, and the CBDS computer subsystem. The contractor operates a subsystem to accept, log, and process data collection forms submitted by the scientific research organizations participating in the bioassay program. Trained and qualified staff operate this subsystem with functions including: a) controls forms, edits and codes, operates and maintains a microfilmed data base; b) maintains automated files for contract information and chemical information; c) converts histopathologic diagnoses into Systematized Nomenclature of Pathology (SNOP) codes for entry into the CBDS data base; d) provides trained personnel to conduct data seminars and training classes on the use of the CBDS; e) provides statistical analysis routines and preliminary analysis reports for bioassay program use; and f) develops Tracor Jitco's Management Information System (MIS).

Major Findings: An operational system to control and process NCI bioassay contractor data from initial data entry to updating the CBDS data base and creating microfiche records for all data processed has been developed and documented in collaboration with NCI and DCRT, NIH. A CBDS chemical file has been developed to maintain current chemical information for all chemicals on bioassay testing. A CBDS contract file has been developed to display administrative, chemical and treatment information for all agents being tested by bioassay contractors. In the area of data processing, over 262,440 data collection forms were processed and entered into the CBDS data base during the current contract period. Data tables have been computerized to display: individual animal pathology, tumor and non-tumor incidence summaries by anatomic site, tissue microscopically examined and tumor incidences, food and weight. Statistical routines have been developed to provide tumor incidence analysis and survival analysis.

Significance to Biomedical Research and the Program of the Institute: Data collected from bioassay studies are important in formulating public policy pertaining to the continued use of the agents tested. It is important that the results of these studies be properly recorded and analyzed. The CBDS is providing these functions.

Proposed Course: The operational system implemented by the contractor is functioning and is proposed to continue during the development phases of the technical reports on the carcinogenesis bioassay studies.

Date Contract Initiated: June 26, 1972.

ERDA-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (Y01-CP-20203) (NIEHS)

Title: Environmental Mutagen Information Center (EMIC)

Contractor's Project Director: Mr. J. S. Wassom

Project Officer (NCI): Ms. Joan W. Chase

Objectives: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and make available chemical mutagenesis information. This information may either deal with the testing of chemicals in one of the many available mutagenicity assay systems or deal with studies exploring the mechanisms of mutagenicity. The information collected for this data base is processed and stored in computer files and can be selectively retrieved and distributed to the scientific community. Copies of all papers cited in the EMIC data base are maintained by the Center in a unique document library.

Major Findings: Approximately 2,000 references were added to the EMIC data base during FY 1977 bringing the total number of references on file to about 19,000. These citations have been indexed with Agent/Organism keywords and Chemical Abstracts Service Registry numbers. Correlations with information available in the NCI publications PHS 149 - Survey of Chemicals Tested for Carcinogenic Activity were made with compounds in the EMIC Agent Registry.

Agents in the EMIC data base have also been cross-referenced to agents appearing in the International Agency for Research on Cancer (IARC) monographs and the Agent Registry of the Environmental Teratology Information Center (ETIC).

The following table summarizes portions of this information:

No. of chemicals in EMIC Agent Registry	No. of chemicals in Agent Registries cross-referenced to EMIC		No. of chemicals common to both files
8,000	ETIC	2,880	1,177
	PHS-149	3,634	1,180

As a result of the wealth of information accumulated in its data holdings and library, EMIC has been called on repeatedly to provide assistance to a number of groups and agencies concerned with the evaluation of chemicals for carcinogenicity. For example, EMIC supplies mutagenicity information to the NCI Chemical Selection Working Group through the Stanford Research Institute and directly to the IARC for use in preparing its NCI-sponsored monographs. The Center assisted the NCI in assessing the utility of short term mutagenicity assays for predicting carcinogenic activity. In this study, a review was made of results obtained from 500 published papers available from the EMIC file which reported on the use of selected non-mammalian assay systems to measure the mutagenic activity of various chemical compounds of special interest to the Institute. The chemicals considered in this review were classified as being either an ultimate, pro- or non-carcinogen. The assay systems considered were those available using Neurospora crassa, Escherichia coli, Salmonella typhimurium, Drosophila melanogaster or the induction of prophage or other episomes in various bacteria. Our review led us to conclude that if properly conducted and evaluated, results obtained with mutagenicity assays from the Ames Salmonella test, and possibly Drosophila (sex-linked recessive lethal test), have merit as indicators or potential carcinogenicity.

EMIC's data base is part of the National Library of Medicine's (NLM) TOXLINE system and was recently included in the Energy Research and Development Administration's (ERDA) RECON system. Anyone with access to either of these two files can now query the EMIC data base directly.

Significance to Biomedical Research and the Program of the Institute: Information on chemical mutagenesis is widely dispersed in the international scientific literature and access to this literature is of prime interest to many workers in the field of carcinogenesis. EMIC's up-to-date and thoroughly indexed data base provides a unique and readily available source for this information.

The processes of mutagenesis and carcinogenesis are complex and are related in their mechanism(s) of action. In the last few years, as more knowledge has accumulated about the structure and metabolic fate of chemicals with respect to their biological activity, a general qualitative correlation between mutagenicity and carcinogenicity has been made. In view of this, EMIC's work has taken on even greater significance to cancer research since the possible use of certain chemical mutagenicity assay systems as prescreens for chemical carcinogens now seems imminent. These short term tests will be used to determine the chemicals to be submitted to more extensive carcinogenesis bioassay systems. This idea has been endorsed by the Subcommittee on Environmental Carcinogenesis of the National Cancer Advisory Board.

Now that the compounds in the EMIC Agent Registry have been correlated to sources of carcinogenesis information, this correlation, along with EMIC's indexed literature collection and document library makes it possible for the program of the National Cancer Institute to respond to its own and other queries for experimental data. This activity helps the NCI and its contractors to fulfill the responsibilities stated in the National Cancer

Act of 1971 which is to -

> collect, analyze, and disseminate all data useful in the prevention, diagnosis, and treatment of cancer including the establishment of an international cancer research data bank to collect, catalogue, store, and disseminate insofar as feasible the results of cancer research undertaken in any country.

Proposed Course: Due to increasing interest in the correlation between mutagenesis and carcinogenesis and in the use of mutagenicity assays as carcinogen prescreens, it seems highly desirable that a data base dealing exclusively with the mutagenicity of chemicals be maintained on a continuing basis.

To this end, EMIC will continue to monitor the international scientific literature. All publications which deal with the evaluatuion of chemicals in the human environment for mutagenicity will be collected and added to the data base. We will continue to index these publications in a manner best suited to meet the needs of researchers and health administrators. Efforts will also continue to make this information as current and as readily available as possible. Acquisition and processing of literature published before 1968 will be pursued to the extent that time permits.

Initial inquiries will be made on the use of pattern recognition programs designed to analyze and make comparisons between data selected from mutagenicity and carcinogenicity studies and to correlate these events with chemical structure and/or other chemical features.

Date Contract Initiated: June 30, 1972

FRANKLIN INSTITUTE RESEARCH LABORATORIES (NO1-CP-43293)

Title: Carcinogenesis Abstracts

Contractor's Project Director: Dr. Bruce H. Kleinstein

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide an up-to-date abstract service for carcinogenesis researchers throughout the world in a format designed to provide under one cover scientific work in the various disciplines which relate to cancer etiology.

Major Findings: The production of Volumes 12 and 13 is completed, and Volume 14 is expected to be complete in May of this year. Volumes 12, 13, and 14 include a Wiswesser Line Notation index and a Chemical Abstracts Registry Number index in addition to subject and author indexes. Close coordination is maintained with NLM and the ICRDB program to make the abstracts available to the CANCERLINE computerized data base as soon as possible, in advance of publication of the printed version.

Significance to Biomedical Research and the Program of the Institute: The multidisciplinary approach used in the study of carcinogenesis needs an information exchange medium which gathers the relevant articles under one cover; this allows for intellectual cross fertilization among the disciplines to produce a more rapid evaluation and use of information in the study of carcinogenesis.

Proposed Course: To continue the publication for an indefinite period of time and to provide abstracts and citations, which will be annotated.

Date Contract Inititated: February 1, 1973

FRANKLIN INSTITUTE RESEARCH LABORATORIES (NO1-CP-55634)

Title: Information Resource

Contractor's Project Director: Dr. Paul N. Craig

Project Officer (NCI): Ms. Joan W. Chase

Objectives: This project provides the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: By use of a basic ordering agreement, it has been possible to structure information and data-handling tasks (which are defined by the requestor, the project officer and the contractor's project director). The 4 tasks assigned this year, include screening the scientific literature for relevant information on selected narrow areas of research, then gathering, collating and evaluating that information.

The four tasks begun or re-initiated this year were: "Current Awareness Services for Nitrosamines Program of DCCP"; "Literature Search for Alkylation of Macromolecules by Carcinogens"; "Information on Carcinogenesis Potency Indexes"; and "Literature Support Services to the Lung Cancer Segment".

Continuing tasks from last year were: "Literature Support for the In Vitro Chemical Carcinogenesis Program"; "Production of an Anatomical Atlas for the European Giant Hamster"; and "Literature Support Services for the Human Tissues Study Section".

Significance to Biomedical Research and the Program of the Institute: All aspects of scientific research have information needs ranging from searching the current literature to preparing critical reviews which are necessary for the optimal development of a research program. There has been a phenomenal increase of published literature in the past several years including a proliferation of journals. Since carcinogenesis research encompasses a number of scientific areas, much time and effort can be expended by an individual investigator in pursuing information needs, some of which can be readily handled and others for which the resources are not available but are necessary for the proper development of research.

This project provides the resources for satisfying information needs of investigators and science administrators in the Carcinogenesis Program in a timely and effective manner.

Proposed Course: The contractor will continue to respond to the information needs of the Program.

Date Contract Initiated: June 30, 1976

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-45608)

Title: Program on the Evaluation of Carcinogenic Risk of Chemicals to Man

Contractor's Project Director: Dr. Lorenzo Tomatis

Project Officer: Dr. Sidney Siegel

Objectives: The International Agency for Research on Cancer (IARC) with support from the National Cancer Institute of the US (NCI) has continued the program on the Evaluation of Carcinogenic Risk of Chemicals to Man. The first aim of this program is to collect, critically analyze and evaluate the existing carcinogenicity data on various groups of environmental chemicals and to disseminate these data in the form of published monographs which are made available to governments, regulatory agencies and interested scientists. The worldwide distribution of the monographs is provided through the distribution service of WHO and in this way they are made available to industries as well as to any interested reader. These monographs are prepared with the help of international groups of experts in various fields of cancer research. The evaluations formulated by such Working Groups have been used as a reliable source of information to governments in many countries in the world. Through the monograph program important chemicals for which adequate data on carcinogenicity are not available, although widespread human exposure exists, have been identified. Information on which chemicals should undergo further testing has also been obtained. Because of the limited facilities available throughout the world for the long-term testing of many chemicals, IARC has also extended the aim of the program by initiating a Survey of Chemicals Being Tested for Carcinogenicity. The outcome of the survey is a Bulletin which reports chemicals presently under test in many of the major institutes throughout the world who carry out such work. Through this survey it is hoped that duplication of effort will be avoided and that communication between investigators increased.

Major Findings: Up to March 1977 fourteen volumes of the IARC monographs have been published and a further volume is in press. In these volumes the carcinogenicity of some 336 chemicals has been evaluated. The number of chemicals for which evidence of carcinogenicity to man or a strong suspicion of carcinogenicity to man exists is twenty-five. For 210 chemicals, definite evidence of carcinogenicity or a suspicion of carcinogenicity in experimental animals exists but case reports and/or epidemiological studies in man are not available or are inadequate. Further testing in animals would appear

desirable in the case of 78 chemicals.

Volume 10 contained monographs on some naturally occurring substances; Volume 11 contained monographs on cadmium, nickel, some epoxides and miscellaneous industrial chemicals; Volume 12 considered some carbamates, thiocarbamates and carbazides; Volume 13 some pharmaceutical substances; Volume 14 asbestos; and Volume 15 (which is presently undergoing final editing) some fumigants, industrial chemicals, 2,4-D, 2,4,5-T and the chlorinated dibenzodioxins.

In March 1976 Volume 6 of the Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity was issued. The Bulletin updates the data contained in previous bulletins and includes 828 chemicals being tested in 89 institutes in 19 countries.

Significance to Biomedical Research and the Program of the Institute: In terms of the US National Cancer Act of 1971, the IARC monographs as formulated by international groups of experts can assist NCI in terms of "collecting, analyzing and disseminating data useful for the prevention, diagnosis and treatment of cancer, including the establishment of an international cancer research data bank to collect, catalogue, store and disseminate, in so far as feasible, the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country".

Proposed Course: The monograph program will be continued and three or four international meetings will be held each year.

In June 1977 a Working Group will consider some further aromatic amines which are used as industrial chemicals, hair dyes or coloring materials.

In October 1977 a further Working Group will consider some nitrosamines and other N-nitroso compounds which have been identified in the environment and for which evidence of carcinogenicity in animals exists. Monographs on nitrosamines and N-nitroso compounds published in Volumes 1 and 4 of the monograph series will be updated. The monograph on polychlorinated biphenyls will also be updated in view of some recent observations in humans.

Also in October 1977 an ad hoc Working Group will be convened to update the criteria on which the evaluation of the carcinogenic risk of chemicals to man is made. The Working Group will be attended by experts in experimental carcinogenesis, mutagenesis, epidemiology, biostatistics and teratology. The outcome of the meeting should be an updated preamble which will give guidance for the preparation of monographs and the formulation of an evaluation which should be more elaborate and constructed in a way to be more useful for public health purposes and regulatory agencies. An assessment of the criteria used to evaluate the significance of the animal data (with particular attention given to the adequacy of the experiments considered) will be needed. Special attention will be devoted to short-term tests now available to screen environmental chemicals for their biological activities in terms of selecting those which need in-depth

testing and also in terms of the possible prediction of health hazards. A very important part of the meeting will be devoted to the assessment of the criteria used to evaluate human data and the weight which should be given to the evidence which might arise from case reports and epidemiological studies. Another issue which will be dealt with is the possibility of establishing a scale of carcinogenic potency. This is of particular relevance if the information given in the monographs is to serve as a basis for regulatory measures.

A further questionnaire has been sent out to update the Survey of Chemicals Being Tested for Carcinogenicity. Information on chemicals which have been put under test since the last inquiry and also the present stage of experiments mentioned in Bulletin No. 6 has been requested.

A further Working Group is planned for February 1978 at which it is planned to consider a group of plastic monomers, co-polymers and polymers.

Date Contract Initiated: May 15, 1975

STANFORD RESEARCH INSTITUTE (NO1-CP-33285)

Title: A Research Program to Acquire and Analyze Information on Chemicals That Have Impact on Man and His Environment

Contractor's Project Directors: Mr. Arthur A. McGee
Dr. Kirtland E. McCaleb

Project Officer (NCI): Dr. Herman Kraybill

Objectives: It is the objective of this 5-year contract to provide support to the Carcinogenesis Program and the National Cancer Program by determining those chemicals with which man come in contact and making available data and information on their structure, production, distribution and exposure level. In accomplishing this objective, information is developed that assists NCI in (1) choosing those compounds having highest priority for entry into the bioassay testing program, (2) identifying trends in the exposure of man to chemicals in his environment, (3) identifying specific subpopulations who may be at high risk due to chemical exposures, and (4) identifying points at which the information accumulated may be best interfaced with the activities of other agencies, both nationally and internationally.

Major Findings: The major effort during the past year has been the support of the Chemical Selection Working Group (CSWG), both recommending and documenting the selection of chemicals for bioassay. To aid the Carcinogenesis Program on a daily basis, a three-person SRI liaison group was established in Bethesda, Maryland in November, 1976.

Work has continued on the expansion of the exposure data bank. Exposure categories under development are agricultural chemicals, adhesives and sealants, and occupational exposure.

The design and programming of the data processing system was completed this year. A thorough documentation of the system was started and is scheduled for completion this summer.

Through this contract, supported by NLM funding, SRI has continued to provide information to NLM's Toxicology Information Program.

SRI continued to support the production of monographs on the evaluation of carcinogenic risk of chemicals to man being produced by The International Agency for Research on Cancer (IARC). SPI assists IARC in the selection of chemicals for study and provides production, use, exposure, occurrence, and analysis information on selected chemicals.

Significance to Biomedical Research and the Program of the Institute: This study provides information on many chemicals of interest both to the Carcinogenesis Program and to other programs concerned with the impact of chemicals on man.

Proposed Course: The project will continue to provide data useful for the selection of compounds to be introduced into the Carcinogenesis Bioassay Program. The information needs of the following activities will be determined and incorporated into the system where feasible: (1) activities in the overall Carcinogenesis Program and in other organizational elements within NCI, and (2) activities within other Government programs concerned with the health hazards arising from exposure to chemicals.

Date Contract Initiated: May, 1973.

SUMMARY REPORT

BIOLOGY OPERATIONAL UNIT

July 1, 1976 through September 30, 1977

The Biology Operational Unit was formed this past fiscal year and consists largely of the activities previously carried out under the Biological Models, Colon and Lung Cancer Segments. The Biology Operational Unit is responsible for developing experimental models for studying the etiology and pathogenesis of the major forms of human cancer. A further objective is the subsegment use of these models for the development of methods to prevent or modify the induction and progression of these tumors. Research areas under investigation include studies on the pathogenesis of cancers of the pancreas, prostate, mammary gland, liver, colon and lung, as well as studies on perinatal carcinogenesis. Investigations are conducted at all levels of biological organization from long-term animal studies through cell and organ culture to the molecular level.

The forms of cancer chosen to be investigated and the basic fundamental concepts of carcinogenesis to be explored are selected with extensive scientific advice from outside expert consultants and from NCI staff as well as with consideration of needs in the field. The development of the program is based on an evaluation of current activities and their impact on program goals, and on new or specific ideas offered by the scientific community, particularly as they result from advances made in new research concepts and methods. The extent of implementation of the program is determined by available human and fiscal resources. Close contact in these endeavors has been maintained with the organ site programs developed in the Division of Cancer Research Resources and Centers. Significant accomplishments of the entire program in this period are described below.

Pancreas Carcinogenesis

As previously reported, the pancreas carcinogenesis program had successfully achieved one of its principal, initial objectives with the development of three potential animal models: (1) methylnitrosourea induction in strain 13 guinea pigs, (2) azaserine in rats, and (3) dihydroxypropyl nitrosamine in Syrian golden hamsters, (the latter obtained in the course of a systematic bioassay and study of substituted nitrosamines). These results justified further exploration in each of these models, the last model in particular showing high promise and considerable extension by program and the scientific community. Other studies were initiated based upon two hypotheses for the mechanism by which a carcinogen can come in contact with the susceptible pancreatic cell: (1) preferential uptake from blood, concentration in pancreatic tissue and excretion through the pancreas ductules, and (2) excretion of conjugated carcinogens in bile through the common bile duct followed by reflux or reverse flow of pancreatic juice and bile constituents into the head of the pancreas where deconjugation could release the carcinogen at the site of the susceptible cell.

The pancreas program is currently organized into four sub-areas, each of which is discussed separately for purposes of this report.

a. Human studies - In the contract at the Mayo Foundation (NO1-CP-55660) the anatomical relationships between the human pancreatic duct, bile duct and duodenum are being examined at autopsy, and this arrangement related to duct cell histology. Analysis to date indicates a trend toward an association of non-common channel ductal arrangements with abnormal pancreatic duct histology. In this contract a canine model has also been developed which allows simultaneous quantification of pancreatic enzyme outputs, duodenal volume flow, and pancreatic and duodenal pressures under physiologic conditions. Of the interesting observations made, one shows the surprising result that mean duodenal pressure can exceed mean pancreatic pressure shortly after feeding. Recent experiments show that reflux of a small amount of duodenal contents into the pancreatic duct can occur under these conditions. This is especially interesting in view of the reported bile reflux in the monkey. (See University of Utah below.)

At the Memorial Hospital for Cancer and Allied Diseases (NO1-CP-43232), detailed studies have been carried out on the cell origin of human adenocarcinoma of the pancreas using light microscopic, histochemical and election microscopic techniques. Results indicate that about 90% of pancreas cancers arise in duct epithelium and may be classified into ten distinctive morphologic types. Marked atypia and carcinoma in situ of pancreas ducts were found in about 20% of patients with pancreas adenocarcinoma, suggesting a significant latent period between the onset of the recognizable neoplastic process and the appearance of invasion and symptoms. The existence of such a latent period should speed efforts to develop a marker(s) to identify cases during the in situ period. Clinical and pathologic study of periampullary carcinoma in this contract has resulted in a new classification to separate duodenal and periampullary tumors from pancreas duct cancers.

The most common type (55%), intra-ampullary tumors, appear to originate in ampullary tissues proper, while exophytic lesions (29%) probably have a duodenal origin and a mixed group of ampullary-exophytic tumors (16%) may have more than one site of origin. The spread of pancreas carcinoma has also been investigated. In duct cell adenocarcinoma (380 patients), peripancreatic lymph nodes, adjacent organs and liver were common sites of tumor extension at surgery and at autopsy with carcinomas of the head of the pancreas tending to spread more locally and cancers of the body and tail more diffusely. Histological examination of pancreas duct cancers showed a high degree of nerve sheath involvement, less vascular involvement and involvement of islets even less. Other studies showed ampullary and duodenal cancers metastasized less frequently and less widely than pancreas duct cancers with fewer lymph nodes involved.

b. In vivo studies - In vivo studies of pancreas carcinogenesis continue in several animal species so as to provide program with a better understanding of model systems. The azaserine rat model (Dartmouth College, NO1-CP-33378)

has been extended to nitroso derivatives of amino acids selected on the basis of tissue localization and mutagenicity tests. Preliminary studies with the methylnitrosourea derivatives of lysine, ornithine, and diaminobutyric acid indicate that these compounds are carcinogenic in rats inducing renal, skin and breast tumors within six months after first treatment. All three compounds have striking effects in the pancreas as well, including the induction of atypical acinar cell nodules. Rats treated with the butyric acid derivative have developed extremely dysplastic foci of acinar cells which appear malignant histologically.

In the contract at Northwestern University (NO1-CP-75876, formerly University of Kansas, NO1-CP-23271) studies continue on the histogenesis of guinea pig pancreatic adenocarcinoma. Pancreatic adenocarcinomas have been found in 29% of inbred strain 13 animals between 28 and 44 weeks of N-Methyl-N-Nitrosourea treatment. On the other hand, treatment with azaserine for 24 weeks has shown neither atypical acinar cell nodules nor carcinoma, while treatment with yet a third compound, 2,2-dihydroxy-di-n-propyl-nitrosamine, for 30 weeks has produced angiosarcomas of the liver in nearly 75% of the guinea pigs but only a single (acinar cell) carcinoma of the pancreas.

At the University of Utah (NO1-CP-55709) a primate model has been developed for assessing biliary pancreatic reflux and its possible significance for human pancreas cancer. These studies which identify appropriate ductal pressure conditions provide the first quantitative demonstration of bile reflux under relatively physiologic conditions through the pancreatic duct of monkey, a primate that has biliary-pancreatic ductal system similar to man. Duodenal-pancreatic duct reflux has also been observed in the dog (See Mayo Clinic above.)

Studies with the Hartley strain guinea pig are nearly complete (University of Illinois, NO1-CP-65843) with no pancreatic tumor formation being observed either in nitrosomethylurea or nitrosomethylurethane-treated animals following partial pancreatectomy. Transplacental exposure to these agents, however, does induce a few uterine tumors in female F_1 progeny.

c. Biochemical and pharmacologic studies - Projects at Indiana University (NO1-CP-55654) and Dartmouth College (NO1-CP-55708) were established to examine uptake, retention, and excretion of a variety of chemical moieties of varying structures. Knowledge of this relationship may contribute to an understanding of organ specificity of pancreatic carcinogens and potential neoplastic agents. It is already well known that amino acids are relatively rapidly absorbed from the blood stream, metabolized in the synthesis of proteins and excreted by the exocrine and endocrine physiological functions of the pancreas. Continuing studies show that several steroid hormones, including estriol, estradiol and progesterone have shown the greatest tissue: plasma ratios, second only to certain amino acids. Retention of these hormones is poor, and the decay curves are more rapid in the pancreas than in the plasma. Amino carboxylic acids, other than the alpha amino derivatives,

show little uptake. The pancreas appears to show a log degree of structural selectivity in uptake of alpha amino acids, provided both free amino and carboxyl groups are present. Labeled polycyclic aromatic hydrocarbons show an initial rapid uptake of radioactivity by the pancreas, followed by a slower wash-out.

At the University of Indiana uptake, retention and excretion of a variety of compounds is investigated in order to determine chemical and structural parameters which may be required for these physiological processes to occur in the pancreas. Modest uptake has been shown in the rat pancreas for the acidic compound benzoic acid, benzo(b)thienyl-3-acetic acid and salicylic acid. Retention of the latter two compounds to some extent appears to occur; chemical tests for benzoic acid, however, indicate no significant retention occurs, suggesting radiolabel retained represents metabolite(s). A variety of basic compounds, including decamethonium, isoniazid, D-and L-methadone and propranolol all show significant pancreatic uptake. Of these, L-methadone and propranolol show a greater tendency for pancreatic retention than their metabolites. A number of basic compounds have been shown to localize also in other tissues.

At the Medical College of Georgia (NO1-CP-55656) the metabolic capacity of the rat pancreas is determined for biotransformation of polycyclic aromatic hydrocarbons (PAH). With 7,12-dimethylbenzanthracene (DMBA) and the Long-Evans strain, in vitro experiments indicate similar pancreatic and hepatic metabolite patterns. Benzo(a)pyrene or phenobarbital-pretreatment of the animals did not induce pancreatic but did induce hepatic metabolism of this PAH. Recent experiments have been carried out on the pancreatic metabolism of radiolabeled DMBA in vivo. Early results indicate that radioactivity in the pancreas remains high at a time (16 hours post-injection) when liver radioactivity has markedly declined. In addition, the percentage of water-soluble metabolites in liver is high at all time periods studied and five to six times higher than that in the pancreas. In other studies using fluorescence microscopy, acinar and ductal tissues were shown to have similar capabilities to metabolize 3-methylcholanthrene to fluorescent products; further, ductal tissue was exposed to greater concentrations of metabolites which came from both pancreatic and hepatic sources.

Biochemical studies at the University of Illinois (NO1-CP-65843, formerly Case Western Reserve University, NO1-CP-23284) have shown DNA damage and repair in the pancreas of Hartley strain guinea pigs following carcinogen administration. Damage and repair caused by methylnitrosourethane and 4-hydroxyaminoguinoline-1-oxide, both in vitro in pancreatic slices and in vivo following a single injection, were assayed on alkaline sucrose gradients using preparations of isolated pancreatic nuclei. Under the conditions used, in vitro damage sustained within 30 minutes of exposure was repaired within two to three hours, while damage sustained in vivo was gradually repaired over a one to two week period.

d. In vitro studies - The potential advantages of in vitro systems for investigations of pancreatic carcinogenesis are being explored in two

contracts. These efforts are entirely devoted to determination of necessary procedures and methods for handling and maintaining pancreas tissues in vitro. At the American Type Culture Collection (NO1-CP-65751, formerly Wright State University, NO1-CP-43231) studies to establish reproducible procedures continue for isolation, maintenance, and identification of pancreatic duct and exocrine cells. Efforts this past year indicate that conditioned media from some primary and established cell lines are capable in short-term experiments of stimulating proliferation of pancreatic exocrine cells maintained as colonial aggregrates in vitro. Other studies have focused on the development of histological, histochemical or histoenzymological markers for guinea pig pancreatic duct cells. Four tests (PAS reaction, methyl green/pyronin Y, alkaline phosphatase, ATPase) appear to be especially useful for differentiating between ductal cells and other pancreatic epithelial elements.

Organ culture of exocrine pancreas at the University of Tennessee (NO1-CP-33369) has shown that explants prepared from 7 to 15 day postnatal rat pancreas can be maintained for periods of at least three weeks in serum-free medium with retention of characteristic morphology and pancreatic specific function as monitored by amylase content and release. Long-term preservation was superior in medium containing glucocorticoid, ascorbic acid and ferrous sulfate. Exogenously-added insulin or glucagon were not required; this coupled with insulin assays of medium suggest that pancreatic islets remain functional throughout the culture period. During this period also, DNA synthesis continues, and pancreozymin stimulates amylase release, both phenomena further suggesting retention of functional physiological processes.

Prostate Carcinogenesis

The development of model systems for studies in prostate carcinogenesis continues to occupy a central position in activities of the Biology Operational Unit. Investigations to this end for nearly forty years have met with minimal success. As part of these efforts, a major new emphasis was begun this past year in the area of normal prostatic epithelial cell culture. The Pasadena Foundation for Medical Research (NO1-CP-65850) and the University of Colorado (NO1-CP-65849) will stress culture of normal, prostatic epithelial cells of human while the W. Alton Jones Cell Science Center (NO1-CP-65762) will focus on culture of epithelial cells from the ventral lobe of rat prostate. The identification of early changes in prostatic epithelium in advance of the development of adenocarcinoma should be facilitated by use of these in vitro systems. The determination of specific markers of preneoplastic or premalignant states, or of markers for early stages of the neoplastic state, requires for an in vitro system detailed knowledge of appropriate culture conditions, which is a major focus of these three contracts.

West Virginia University (NO1-CP-75887) recently initiated studies on the relationship between age and hormone environment and the onset

of adenocarcinoma of the prostate. Examination will be made of the hormonal condition of aging rats and its effect on ventral prostate gland transplants as well as effects on the in situ organ. Another project at West Virginia University (NO1-CP-43230) using the cleared mammary gland fat pad of Fischer rats as a site for explants in studying non-steroidal hormone influence on prostate cell proliferation has recently been completed. Results indicate that the age of the donor prostatic tissue, the age of the host animals, as well as the duration of the post-transplantation interval influenced the survival of the prostate transplant. These studies suggest that the in situ prostate's biochemical and morphological aspects can be compared with a transplanted prostate, and that these aspects can be investigated in young (6-12 weeks) and old (54-90 weeks) hosts and donors.

Efforts continue at the University of Michigan (NO1-CP-55720) to define the cultural requirements for canine and human prostate organ cultures. Conditions for long-term culture have yet to be identified; nevertheless, canine prostate can be cultivated in a well-maintained state for 10 days in vitro if the donor dog has been treated with methylprednisolone before prostatectomy, and if the explants are exposed to vitamin A in culture. The contract with Hazleton Laboratories, Inc. (NO1-CP-23287) investigating the possible induction of prostatic adenocarcinomas in dogs given direct intraprostatic inoculations of chemical carcinogens has been completed. Methylcholanthrene (MCA) has induced fibrosarcomas in 39% of sexually immature dogs injected and transitional cell carcinomas in animals treated at 1-2 years of age. However, no adenocarcinomas have been observed due to injection of MCA, and no tumors at all due to N-methylnitrosourea, cadmium chloride or N-methylnitrosoguanidine.

Prostate androgen metabolism and its receptors in rodent, canine, and human prostate has been the subject of studies at the Southwest Foundation for Research and Education (NO1-CP-33379). These studies are of particular interest and importance since, as noted previously, spontaneous adenocarcinomas of the ventral prostate of the AXC rat have been detected with a frequency of 70 percent in rats greater than 30 months of age. In contrast to the rat, testosterone metabolic potential of dog prostates has been shown to have an age-associated increase in reductive processes. Similarly, the metabolites of testosterone of human benign prostatic hypertrophy (BPH) resemble that of the aged dog, presumably undergoing BPH itself as it ages. Unlike the canine species, however, a potential exists for increasing oxidative metabolites from human BPH by use of hyperphysiological levels of testosterone. Human adenocarcinoma is remarkably oxidative, simulating ventral prostate metabolism in aged rats. Consequently, the differences in AXC rats (adenocarcinoma prone) and dogs (BPH prone) may provide the key to the differences (factors) which induce human prostates (BPH and adenocarcinoma prone) to develop, as age increases, into either BPH or cancer. These results further support the hypothesis that changing endocrinology of the aging host is intimately associated with BPH and adenocarcinoma.

Mammary Gland Carcinogenesis

Studies on the synergism of estrogens and radiation in the induction of mammary gland carcinogenesis have been under investigation for a number of years. During this past fiscal year, two new projects have been initiated to examine possible synergistic effects of repeated low-dose irradiation and estrogens on mammary gland carcinogenesis in rats (Alton Ochsner Medical Foundation, NO1-CP-65764; ERDA-NCI Interagency Agreement, Brookhaven National Laboratory, YO1-CP-60219). These new projects may provide important information regarding the risk for breast cancer induction from repeated mammographic examinations. They will complement similar ongoing projects with these institutions (NO1-CP-43229; YO1-CP-30213), and the Organization for Health Research (TNO) (NO1-CP-33330). Findings from these projects to date include: 1) in the AXC rat strain, synergism with diethylstilbestrol (DES) implants occurs with X-rays and with neutron radiation; 2) this interaction has not been observed in the Sprague-Dawley strain; 3) in the AXC female rat, DES appears to be additive (rather than synergistic) with dimethylbenzanthracene; and 4) in three different rat strains of control, X-ray, and 15MeV neutron irradiated groups, preliminary observations indicate that irradiation alone (without added estrogen) causes a greatly increased frequency of benign tumors with little or no increase in histologically malignant tumors.

At the University of Nebraska (at Lincoln) (NO1-CP-33289), research to determine relationships between chemical carcinogen-induced nodule-like alveolar lesions (NLAL) and tumorigenicity is continuing. In the coming months, the tumorigenic potential of NLAL and/or other latent transformed cells will be investigated in glands treated with dimethylbenzanthracene.

The rhythmometry studies of hormones in Japanese and North American women at the University of Minnesota (NO1-CP-55702) should aid in determining relationships of rhythmicities affecting individual hormone concentrations in high and low risk populations. Radioimmunoassays which have been completed and analyzed thus far show a significant relationship between circadian rhythm in four Minnesota subjects and cortisol, thyroxine, and prolactin.

Perinatal Carcinogenesis

Tissue exposure to carcinogens during perinatal development may have significant carcinogenic consequences, some of which may not become evident until the post-pubertal and adult periods of life. Three contracts in the Biology Operational Unit investigate this important area. At the University of Colorado (NO1-CP-75875, formerly Stanford University, NO1-CP-55649), tissue interactions in induction and perpetuation of hormonally-induced permanent cellular alterations are under investigation. Tryptic separation and recombination methods

with ultrastructural, immunocytochemical, and other studies are utilized to determine interaction mechanisms in normal, abnormal, and neoplastic growth and differentiation of the vagina. Earlier results from this project indicated that estrogen exposure appeared to cause permanent alterations in the developmental properties in vaginal epithelium and stroma.

The development of a nonhuman primate model to demonstrate transplacental chemical carcinogenesis continues at Meloy Laboratories, Inc. (NO1-CP-55613). Earlier findings have shown that 1-ethyl-1-nitrosourea (ENU) produces lytic damage to neuronal and glial precursor cells of the subependymal germinal layers of the cerebral hemispheres in fetal monkeys. Three other age groups of patas monkeys have now developed tumors following exposure to ENU. These tumors appear shortly after birth and resemble those associated with pediatric neoplasms in man. Both these characteristics of the nonhuman primate model differ from rodent models in which transplacental exposure to carcinogen results in tumor development in young adult life of tumors predominantly of adult morphology.

The Aichi Cancer Center Research Institute (NO1-CP-55650) is studying the nature of permanent alterations in the vagina in mice and the coagulating gland in rats, and ovarian dysgenesis and tumorigenesis that result from neonatal thymectomy in mice. Investigations seek to determine whether a correlation exists between the grade and severity of hyperplastic vaginal alterations induced by neonatal estrogen treatment and the populations in the tissue of two types of basal cells which are distinguishable since only one type shows a response to a secondary estrogen treatment. In other studies, preliminary data from estimated estrogen receptor levels in vaginal tissues of mice treated neonatally with estrogen suggests that this treatment causes no significant changes in nuclear or cytoplasmic receptor levels. Neonatal castration and neonatal treatment of male rats with DES or estradiol benzoate generated papillary epithelial bladder growths.

Carcinogenesis in Human Tissues

The aim of this program is to make new in vitro carcinogenesis methods directly relevant to the human situation. Although animal studies are performed when indicated, the emphasis is on utilizing human tissues and cells, obtained in compliance with existing legal requirements. Normal and malignant tissues are obtained at autopsy or surgical intervention and are examined in organ culture for maintenance of morphological and cytochemical integrity. The Veterans Administration Hospital (Washington, D.C.) (YO1-CP-60204) is now supplying bronchial and parenchymal tissues from patients undergoing lung resection for tumors and other diseases, and, in addition has initiated morphological studies on normal, premalignant, and malignant respiratory epithelium.

The project at Biotech Research Laboratories, Inc. (NO1-CP-55640) seeks to establish criteria for in vitro carcinogenesis endpoints which correlate with tumorigenic potential. This work has resulted in the development of a cell aggregation assay to assess the transformed status of a cell population; it has been found that the survival ability of some transformed cells in the aggregate form correlates with colony formation in soft agar and with tumorigenicity in nude mice and syngeneic hosts.

The Children's Hospital of Los Angeles (NO1-CP-55641) has been investigating fibrinolytic activity in malignant transformation in vitro. Collaborative studies indicate that an increase in plasminogen activator could be an early marker for transformation; all human neuroblastoma cell lines developed in this project show high levels of this activator.

Studies of carcinogenesis in human bronchial tissues at Litton Bionetics, Inc. (NO1-CP-43274) utilize the athymic nude mouse as host animal for xenotransplanted human tissues previously exposed to carcinogens and/or anticarcinogens in organ culture. At the University of Maryland (NO1-CP-43237), human bronchial epithelium, pancreatic duct, breast, and colon tissue are acquired, maintained in explant organ culture and studied in great detail by ultrastructural and cytochemical procedures. A new histogenetic classification scheme has been developed, and the results of carcinogen binding to bronchus in vitro is being correlated with tumor type and with epidemiological data obtained from a questionnaire and medical records of patients.

Resources and Other Projects

A number of resource contracts provide capabilities to the carcinogenesis research collaborative program. Two projects initiated this fiscal year provide microscopic and autoradiographic technology (Litton-Bionetics, Inc., NO1-CP-65847 and Experimental Pathology Laboratories, Inc., NO1-CP-65763). Animal breeding at Harlan Industries, Inc. (NO1-CP-55647) is discussed under the Interdisciplinary Programs and Resources Operational Unit. A major resource for laboratory services is provided by Microbiological Associates, Inc. (NO1-CP-02199); 42 individual experimental protocols designed jointly by NCI and the contractor have been employed in collaborative studies during the past year.

Biochemical and morphologic components of hepatic carcinogenesis are now being investigated at the University of Toronto (NO1-CP-75879) [formerly Temple University (NO1-CP-33262)]. The objective of this newly-initiated project is to formulate rapid in vivo bioassay procedures for chemical carcinogens and for identification of early preneoplastic cells in intact animals.

Colon Carcinogenesis

Epidemiological studies have been pursued at the Kaiser Foundation Research Institute (NO1-CP-33215) and the Medical College of Georgia (NO1-CP-43282) to determine the relationship of diet (animal protein,

saturated fats, foods treated with preservatives, refined carbohydrates, high fiber content) to the occurrence of colorectal cancer in the human. Data analysis by the Kaiser Foundation shows that no relationship appears to exist between frequency of beef consumption and colorectal cancer. The frequency of consumption of foods treated with nitrite preservatives, or of foods high in saturated fats, or of meat in general shows statistically nonsignificant positive associations with colon and rectosigmoid junction cancer; statistically nonsignificant negative association with colon cancer was shown for foods high in unrefined carbohydrate or roughage. Statistically significant positive associations have been shown for the nondietary traits of nulliparity in women and for diarrhea. Studies at the Medical College of Georgia are still in the data-gathering phase.

Analyses of the microbiological flora and neutral sterol composition of feces from individuals classified according to their statistical risk of developing colon cancer are being performed at the Virginia Polytechnic Institute and State University (NO1-CP-33334, NO1-CP-55685), the University of California at Los Angeles (NO1-CP-33380), and the University of Missouri (NO1-CP-33335). These investigations attempt to determine if certain microbiological or chemical markers for risk exist, as well as if a specific microbial species may play a unique role in the carcinogenic process. A number of observations of interest have been made, but firm conclusions or hypotheses are still premature. A change in diet itself does not necessarily alter the microbial genera and species found in the feces. A low risk group appears to differ in relative quantities of different types of aerobic and anaerobic microorganisms from a high risk group. However, all types seem to be present in some degree irrespective of risk classification. High meat consumption increases the proportion of anaerobes. This is not mediated through high cholesterol intake since evidence indicates no increase in cholesterol or its metabolites was found in the excreted solids.

The association of Clostridium paraputrificum with risk group classification appears to be strengthened by measurement of quantitative levels of this bacterium in vegetarians and meat consumption populations. Studies on isolation and evaluation of the activity of this microbe appear warranted. Microorganisms have been isolated which require cholesterol for growth. Two major degradation products, coprostanol and ethyl-coprostanol, seem to be more highly esterified to long-chain fatty acids in a low risk population.

The Memorial Hospital for Cancer and Allied Diseases (NO1-CP-43366) has developed results suggestive of a hereditary factor in skin fibroblasts in familial polyposis, phenotypically characterized by in vitro growth morphology, a possible marker for latent inherited polyposis. Fibroblasts from polyposis subjects were 100-1000 fold more susceptible to transformation (focus formation) with Kirsten murine sarcoma virus than normal skin cells. Studies of chromosomal protiens of fibroblasts

by electrophoresis in SDS gels revealed differences between chromosomal proteins from desmoid tumor and those from fibroblasts derived from flat skin; and studies of distribution of actin cables in fibroblasts from polyposis and control groups suggest that fibroblasts from normal individuals contain more actin cables than those from polyposis groups. It was also determined that a high amount of cholesterol, and low levels of coprostanol and coprostanone, were excreted by individuals with familial polyposis.

The American Health Foundation (NO1-CP-33208) continues to emphasize the role of diet in experimental studies of colon carcinogenesis. In germfree and in conventional rats, deoxycholic acid, cholic acid, and chenodeoxycholic acid were found to be colon tumor promoters, extending previous findings that taurodeoxycholic and lithocholic acid promoted colon cancer induction in conventional animals.

In contrast to these findings of promoting capability in the descending part of the large bowel in rats, a series of bile acids applied to the skin of mice together with benzo(a)pyrene in a classical test of co-carcinogenicity failed to demonstrate a cocarcinogenic effect. In fact, an inhibiting effect on the carcinogenicity of benzo(a)pyrene was found in some cases, including some neutral sterols.

Other studies at American Health Foundation have shown that the β-glucuronidase activity in cecal contents of rats fed high fat-high protein diets was significantly higher than that from rats fed normal fat-normal protein diets. This is in contrast to the β-glucuronidase activities of the small intestine and colon mucosa which showed no differences in rats fed these diets. Still other studies are developing specific and sensitive analytic systems to measure fecal excretion of benzo(a)pyrene metabolites in rat and human stools.

The University of Oxford (NO1-CP-23215) project on data collection and analysis for the possible determination of disease linkage has resulted in several statistically significant associations between colon cancer and cancer in other tissues and organs.

Lung Carcinogenesis

The Lung Cancer Program of the Biology Operational Unit is devoted to studies of the pathogenesis of lung cancer and its prevention. The ultimate goal of the Program is the prevention of development of lung cancer in man. A variety of experimental techniques are used in many investigations, ranging from long-term animal studies to short-term morphological and biochemical experiments. All of these efforts are coordinated to establish a correlation between animal models and human lung cancer, and to develop a targeted approach to the inhibition or prevention of this most prevalent form of fatal cancer in man. The need for such a program to lessen the incidence of this widespread, lethal disease scarcely requires further comment. During the past year major investigations in the areas of in vitro carcinogenesis in

respiratory epithelium, and in several aspects of retinoids research have further developed. Significant accomplishments of the entire program in this period are described below.

At the Oak Ridge National Laboratory (ERDA-NCI Interagency Agreement [YO1-CP-50200]) the pathogenesis of lung cancer is being investigated in a number of long-term animal studies that are of direct relevance to the human disease. The potential cocarcinogenicity of several irritant gases, that are common combustion products (such as nitrogen dioxide) or are prevalent in the gas phase of tobacco smoke (such as formaldehyde), is being tested in rat and hamster lung tumor models. The toxic response of the respiratory tract epithelium to these irritants is also under investigation. Findings are available from the NO_2 studies which indicate that in seven different nitrogen dioxide exposure groups in hamsters, the gas does not measurably enhance pulmonary carcinogenesis induced by intratracheal intubation of benzo(a)pyrene.

In continuing studies of the modulation of development of respiratory tract neoplasia by retinoids, it was found that NMU-induced tracheal tumor incidence is decreased 25-35% by the highest nontoxic doses of two retinoids employed. At lower carcinogen doses, larger inhibitory effects of the retinoids are indicated in ongoing experiments.

The in vitro tracheal organ culture system previously developed has been used in studies of initiation-promotion in respiratory tract epithelium. It has been found that respiratory tract epithelium can be transformed in vitro with chemical carcinogens and that the promotor TPA stimulates DNA synthesis in a dose-dependent manner.

The tracheal transplant system previously developed to investigate the role of cofactors in respiratory tract carcinogenesis is being used in a number of investigations: a) Dose-response studies for the carcinogenic polycyclic hydrocarbons, benzo(a)pyrene and dimethylbenzanthracene; b) studies on the age-specific susceptibility of tracheal epithelium; c) histogenesis experiments on the fate of various epithelial lesions induced by DMBA; and d) biochemical studies of the glycoprotein moieties of mucus.

At the Harvard University School of Public Health (NO1-CP-33273), the synergism between radiation and chemical carcinogens in causing lung cancer is being studied. Such synergism has been implicated in the high incidence of occupational lung cancer in uranium, hematite and flurospar miners; and possibly also in the smoking population. Significant investigations on the distribution of carcinogens in the respiratory tract combined with long-term carcinogenesis studies have shown that the segmental distribution of lung cancer induced in hamsters by the intratracheal instillation of either polonium-210 or BP adsorbed onto hematite particles, as well as BP administered in gelatin-saline suspension, can be related to the transport and localization of each agent in the respiratory tract. It has been found that mean alpha radiation doses to the lung from polonium-210 of only 15 to 75 rads produces lung cancers in hamsters. Cell culture studies examining various parameters of malignant transformation have shown a synergistic action

between X-rays and BP, with preliminary data suggesting an important role for cell proliferation in this synergism.

At the New York University Medical Center (NO1-CP-33260), the major emphasis is on the identification of hazardous materials which may pose a serious threat to man as respiratory carcinogens. Great effort has been spent in the design and establishment of an inhalation chamber facility which can accommodate over 1000 animals. Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. Among the important substances which have been found to be carcinogenic or cocarcinogenic for the respiratory tract are calcium chromate dusts, sulfur and nitrogen dioxide, dusts formed from polyurethane foam (workers in the building trades industry may be exposed to this type of dust), and bischloromethyl ether, a common industrial chemical. Results in long-term animal studies show that croton oil inhalation following MCA or BP intubation in hamsters results in an increase in respiratory tract cancer incidence compared to that in animals receiving carcinogen alone. Chronic inhalation studies will begin on chrome process residue actually present in commercial chromium refining and on polyurethane foam as soon as the respective industries can make materials available. In lifetime exposures to dimethylcarbamoyl chloride at 1 ppm, 94 of 98 rats incurred squamous cell carcinomas of the nasal cavity. This result suggests a high level of carcinogenic activity for this compound, which represents a new industrial hazard. Epichlorohydrin, another widely used industrial intermediate, has been tested in rats with the result that an early warning report of the hazard of this material has been submitted. Studies on combined sulfur dioxide/arsenic trioxide to determine a tolerated dose for chronic exposure are in progress.

Since many projects in the lung cancer program involve long-term animal studies in defined models of lung carcinogenesis and modulation of epithelial carcinogenesis in other systems, a contract provides for the supply of animals treated with respiratory and other carcinogens (IIT Research Institute [NO1-CP-43289]). This contract prepares and delivers Syrian golden hamsters and rats treated under protocols leading to carefully controlled conditions for the initiation of preneoplastic lesions and tumors of the respiratory tract and bladder. The various treatment regimens and periods of treatment are used to produce a variety of preneoplastic lesions.

At the IIT Research Institute (NO1-CP-23292) major long-term animal studies continue on the modulation of epithelial carcinogenesis by retinoids. It has been found that retinyl acetate and the synthetic retinoid, retinyl methyl ether, significantly reduce dimethylbenz[a]anthracene-induced benign and malignant breast tumors in the rat. Further studies have shown that N-nitrosmethylurea induces a high incidence of mammary adenocarcinoma in the Sprague-Dawley female rat and that the development of mammary cancer is markedly inhibited in this model by retinyl acetate.

Studies of retinoid inhibition of bladder carcinogenesis have also been carried out. It has been found that 13-cis retinoic acid reduces the incidence of butyl-(hydroxybutyl)-nitrosamine (HO-BBN)-induced bladder cancer in the Fischer rat by 50%, and decreases the severity of the lesions. Other retinoids are under evaluation in the Wistar rat (HO-BBN-induction), and improvement of the rat model and development of a mouse bladder carcinogenesis model are in progress.

Since a significantly large number of deaths from lung cancer in man are caused by oat cell carcinoma (which is a particularly invasive and metastatic type of tumor), a new effort was initiated this year with emphasis on the development of an animal model for this particular respiratory tract cancer (Dartmouth College, NO1-CP-65776).

To effectively pursue many of the above studies on lung tissue from both man and animals, improved cytological methods, such as autoradiography, are being conducted at the VA Hospital in Tampa (YO1-CP-55625). The technique of light microscopic autoradiography of the respiratory tract has been improved, so that the actual amount of radioactivity in cellular and subcellular compartments of respiratory epithelium can be quantitatively measured. It has been found previously that intratracheal administration of BP, as well as vitamin A deficiency, causes an increase in cellular proliferation in respiratory epithelium, as quantitated by thymidine labelling. In addition, it has been found that vitamin A deficiency causes loss of ciliated cells and an increase in the number of basal cells in respiratory epithelium. High doses of vitamin A cause an increase in ciliated cells.

The in vivo cellular and subcellular localization of ^{3}H-BP in tracheal epithelium of normal and retinoid-deficient hamsters has been studied. Total radioactivity in deficient hamsters was greater than in retinoid sufficient hamsters in the basal and suprabasal regions of squamous metaplasia. However, no significant differences were observed in specific activity of nuclear or cytoplasmic compartments between normal animals and either squamous metaplastic or nonmetaplastic areas of deficient animals.

A major study on in vivo cell kinetics in adult hamster tracheal cells has employed many separate methods for determining cell cycle parameters: fraction of ^{3}H-TdR-labeled mitoses; double-labeling with ^{3}H and ^{14}C-TdR; and grain counts over interphase nuclei as a function of time following a single ^{3}H-TdR injection. Quantitative autoradiographic procedures were employed. Ciliated cells were not labeled nor observed in mitosis. Definitive values have now been established for basal and mucus cells for lengths of S, G2, M, G1, cycle time, growth fraction, labeling index, and mitotic index. The fraction of cells dividing very slowly or "resting" (GO) was also calculated for basal and mucus cells; and the life-spans of these cells estimated (basal 104 days; mucus 119 days; ciliated about 130 days). Findings in these studies suggest

that ciliated cells arise by differentiation of mucus cells rather than of basal cells. These studies of cell kinetics for tracheal epithelium provide a basis for measurement of biological effects of carcinogens and conditions inhibiting carcinogenesis.

Investigations are pursued in two projects devoted entirely to the study of respiratory carcinogenesis in vitro. At the State University of New York at Stony Brook (NOI-CP-33361), methods have been developed for large-scale, long-term organ culture of rat tracheal tissue. As many as 300-500 cultures can be prepared at a time and under suitable conditions grown for periods of months. Cultures are uniform in appearance and in ability to respond to various stimuli by cellular repair and by mitotic activity. A number of types of media have been investigated in studies of epithelial growth and maintenance of differentiation. Serum was shown to facilitate migration of epithelial cells as a continuous flattened sheet.

A series of protocols for exposure of cultures to carcinogens were investigated. Continuous exposure to BP or periods of exposure alternating with periods of carcinogen-free medium was found to result in shortening of the life of the culture in rough proportion to the concentration of carcinogen. Cultures exposed continuously for two weeks to BP, and then removed to carcinogen-free medium developed areas of focal hyperplasia. These areas persist for at least six weeks, and are reversed or prevented from appearing by retinyl acetate, retinoic acid and three of four analogues of retinoic acid tested. Cultures showing focal epithelial hyperplasia at the time of implantation had reverted to normal histology. Ultrastructural, biochemical, and cell cycle kinetic studies are performed to identify markers of malignant transformation before diagnostic histologic changes appear. It has been observed that the cells of carcinogen-induced hyperplastic regions secrete plasminogen activator and have abnormal plasma membrane structure. Lanthanum staining and freeze fracture has revealed extensive atypical junctions in the epithelium. Comparisons are made with untreated cultures, normal tracheal epithelium, regenerating tracheal epithelium, tracheas treated in vivo with BP, and tracheal tumors arising in vivo. Companion monolayer culture studies are performed of cells derived from carcinogen-treated cultures.

In the second project, organ culture studies at the University of Vermont (NOI-CP-33360) have systematically explored various chemically-defined media and the effects of serum, insulin, retionids, and adrenal corticosteroids on long-term cultures of hamster tracheal epithelium. Explants maintained in "complex" chemically defined medium with serum exhibit marked epithelial hyperplasia and dedifferentiation, whereas tissues maintained in minimum basal medium with serum retain a defferentiated appearance. Squamous metaplasia develops in the absence of serum or retinoids. In a retinoid-free medium non-essential amino acids, especially serine and glutamic acid, promote and strikingly enhance the development of squamous metaplasia.

In other studies, anaplastic carcinomas have been regularly induced in hamsters by implants of tracheal organ cultures exposed to inorganic dusts and 3-methylcholanthrene (MCA). The cocarcinogenic effects of ferric oxide and crocidolite asbestos have been compared: with asbestos tumors can be induced with 10- to 20-fold lower concentrations of MCA than with ferric oxide. This corresponds to less than 0.1 µg MCA per 4 mm^2 of epithelial surface.

Retinoids added to cultures of tracheal explants exposed to amosite or crocidolite asbestos inhibit augmented DNA synthesis in the tracheal epithelium without inducing degenerative mucosal changes.

Other experiments employing both contractor's procedure of exposing cultures to carcinogen bound to Lycra fibers and an adaption of the Oak Ridge tracheal graft procedure indicate that a continuous carcinogenic stimulus must be applied to the respiratory epithelium in vivo in order to induce malignant transformation.

Since it is well established that many carcinogens (including those which affect the lung) must be metabolically activated before they can exert their carcinogenic effects, a major effort has been developed in this area. Such research has long-term potential to find effective means to block the conversion of inhaled or ingested carcinogens to metabolically active forms, and thus to inhibit the development of lung cancer. Three contracts study polycyclic hydrocarbon metabolism in the respiratory tract.

At the University of Minnesota (NO1-CP-33364), efforts are focused on the role of anti-oxidants, such as butylated hydroxyanisole (BHA), in the inhibition of pulmonary neoplasia resulting from administration of polycyclic hydrocarbons. It has been found that BHA alters the metabolism of BP by the microsomal mixed function oxidase system under conditions which inhibit BP-carcinogenesis. Use of microsomes from mice fed BHA results in significantly less binding of BP metabolites to DNA than with control microsomes. This reduction in binding occurs with microsomes from all eight strains of mice investigated. Further, analysis of BP metabolites by high pressure liquid chromatography from control and BHA-fed microsomal systems indicates BHA results in profound alterations of the metabolite pattern. Such alterations can be divided into two categories both of which result in a decrease in the carcinogenic effect of BP: first, an increase occurs in 3-hydroxybenzo(a)benzene, a metabolite of detoxification; and second, a decrease in epoxidation which is a process necessary for the metabolism of benzo(a)pyrene to its ultimate carcinogenic form. Moreover, epoxide hydrase and glutathione-S-epoxide tranferase activities were similar for both enzymes in control and BHA-fed microsomes, indicating that alterations in these activities are not responsible for the observed major differences in metabolite patterns.

At the University of Stockholm (NOl-CP-33363), efforts are directed at defining the relationship between the microsomal enzyme system, aryl hydrocarbon monooxygenase, and lung cancer. A sensitive and convenient radioactive assay for BP monooxygenase has been developed and employed in studies of kinetics, inhibition, and induction of this enzyme system and the nature of its products; and a new fluormetric assay for this enzyme has been applied to rat lung tissue for similar studies. A method for removing hemoglobin from lung microsomes by sepharose chromatography has been developed, and the spectral properties of cytochrome P-450 in these microsomes investigated. The isolation and characterization of a partially purified preparation of lung cytochrome P-450 itself has been achieved.

Rat lung microsomes have been fractionated and a pure lung microsomal fraction isolated which consists almost exclusively of rough vesicles. These rough microsomes contain about 2/3 of the activity of both NADPH-cytochrome P-450 reductase and BP monooxygenase. The products of BP metabolism by total, rough, and smooth microsomes from the liver and from the lung of rats both before and after methylcholanthrene induction have been characterized using high pressure liquid chromatography. A sensitive modified assay for epoxide hydrase has been developed and this activity characterized in lung tissue; a more sensitive assay for glutathione-S-epoxide transferase has been developed. Levels in lung of glutathione and enzymes of its metabolism have been determined. It has been shown that lung microsomes metabolize 3-hydroxybenzo[a]pyrene to a product which binds covalently to DNA, and that liver nuclei metabolize BP to DNA binding products. The nature of conjugation and its relative importance in BP metabolism by isolated, intact hepatocytes has been studied.

Since the aryl hydrocarbon monooxygenase system is an extremely complex linkage of various molecular components, the investigators at the University of Texas (Southwestern Medical School at Dallas) (NOl-CP-33362) are analyzing the role of the various molecular components in lung microsomes. Several chemical and immuno-chemical inhibitors of the NADPH-cytochrome P-450 reductase dependent reactions have been used to investigate the normal and carcinogen-induced cytochrome P-450-dependent monooxygenases in the lung. Results indicate that this enzyme is the flavoprotein reductase of cytochrome P-450 in lung hydroxylation reactions, donating at least one electron to cytochrome P-450 when either of the two reduced pyridine nucleotides NADPA or NADH is used as a source of reducing equivalents. Use of chemical inhibitors of BP hydroxylase or biphenyl hydroxylase show that the lung contains a constitutive aromatic hydroxylase which is quite active, but does not hydroxylate BP well. A 3-methyl-cholanthrene inducible aromatic hydroxylase exists which is P-450-dependent and which appears to hydroxylate BP but does not hydroxylate biphenyl. These results indicate that two types of cytochrome P-450-dependent aromatic hydroxylases exist in lung, but only the inducible hydroxylase appears to be capable of metabolizing BP.

A cytochrome P-448-dependent substrate has been developed. This substrate, ethoxyresorufin (7-ethoxyphenoxazone), has a high degree of specificity for carcinogen-induced cytochrome P-448. The time course of induction of BP hydroxylase and ethoxyresorufin deethylase activities have similar high sensitivities to certain inhibitors such as 7,8-benzoflavone. The assay for deethylase activity is simple and very sensitive, and has already found application in assessing the state of induction of certain tissues.

Studies of BP metabolism have been initiated using the important recent application of high pressure liquid chromatography to metabolite identification. Metabolite profiles from lung and liver are investigated under varying conditions of induction and inhibition of the BP monooxygenase system. Other studies using electron spin resonance coupled with gas chromatography-mass spectrometry support the suggestion that certain BP phenols can be oxidized, either chemically or enzymatically, to yield anion products with free radical signals.

It is apparent that a great deal of important basic biochemistry remains to be done on this problem, but the results are of great importance to our understanding of the initiation of lung cancer in man. Meetings are held frequently by the three groups of investigators working on this problem of polycyclic hydrocarbon metabolism, with a maximal sharing of information.

Since retinoids have such a profound effect on cellular differentiation in respiratory and other epithelia, and since they have been shown to be capable of inhibiting carcinogenesis in this epithelia, the biochemical bases of these phenomena have been investigated, with the long-term goal of achieving more effective use of retinoids in cancer prevention.

Since the intrinsic toxicity of natural vitamin A is a limiting factor in usefulness in cancer prophylaxis, studies are underway to identify new synthetic analogs (retinoids) that will be more potent and less toxic than the parent substance. At the Southern Research Institute (NO1-CP-22064), such potential analogs are being evaluated, by both morphological and biochemical techniques. These assays have the important feature of providing rapid test data on unknown compounds. The morphological effects are being evaluated in organ cultures of chick skin and mouse prostate, while biochemical studies have employed tissue from both mice and hamsters (liver and lung). In the organ culture assays, several modifications of the ring portion of the natural vitamin A molecule have been found to be as potent as the parent compound in inducing mucus metaplasia of chick skin and two cyclopentenyl analogs of retinoic acid are approximately 10-fold more effective than retinoic acid. The organ culture system for mouse prostatic tissue has become firmly established, and has shown that the induced hyperplasia in explants cultured in the presence of 3-methylcholanthrene or N-methyl-N^1-nitro-N-nitrosoguanidine can be inhibited or reversed by retinoic acid. Retinoids have been found which are more active

than retinoic acid in reversing induced cellular proliferation in this prostatic system: a trimethylmethoxyphenyl and two cyclopentenyl analogs of retinoic acid as well as ether, hydrazone and oxime derivatives. Retinoic acid inhibits testosterone-induced hyperplasia in this system.

A protein which binds retinoic acid and retinoids with a free carboxyl group is present in skin, brain, testes and ovaries of rat, mouse and chick embryos, and perhaps also in other tissues. Such a protein may figure importantly in a function similar to hormone receptors. It is present in some transplantable lung and colon tumors. Various tissues of mice were surveyed for enzymes capable of hydrolyzing retinoic acid esters. In all tissues examined, retinoic acid esterase activity was present. A survey of thirteen retinoids for destabilization of lysosomes from mouse liver and L-929 fibroblasts in culture, and for toxicity to L-929 and human KB cells in culture, found no significant correlation between destabilization and toxicity. Some retinoid compounds inhibit an oxidative reaction catalzyed by a constitutive enzyme in lung microsomes and leading to irreversible binding of BP to macromolecules. Since this reaction was also inhibited by butylatedhydroxytoluene, an anticarcinogenic agent, the reaction may be involved in carcinogenesis by BP. A quantitative procedure for quantifying retinoic acid in biological fluids has been developed.

Two new projects have been initiated this year in the critical area of establishing markers for evaluation of preneoplastic lesions in the respiratory tract (Baylor College of Medicine, NO1-CP-65837; University of Chicago, NO1-CP-65777). These efforts are directed at establishing objective parameters for assessing the status of preneoplastic lesions using advanced spectrophotometric and computerized procedures in cytologic evaluation.

In summary, the Lung Cancer Program is pursuing a coordinated program of studies at the human, animal, cellular, and molecular level, to investigate the factors which are involved in the cause and prevention of lung cancer. It is hoped that within this coordinated framework there will be the most rapid dissemination and utilization of new information obtained at the basic levels, and that we thus may hopefully be able to shorten the time before we achieve the desired goal of prevention of lung cancer in man.

AICHI CANCER CENTER RESEARCH INSTITUTE (NO1-CP-55650)

Title: Role of Hormonal Induction of Permanent Cellular Alterations during Perinatal Exposure on Carcinogenesis

Contractor's Project Director: Dr. Yasuaki Nishizuka

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: (1) To study the nature of permanent alterations of the vagina in mice and the coagulating gland in rats resulting from perinatal exposure to steroid hormones, and their relation to carcinogenesis; and (2) to study the mechanism of ovarian dysgenesis and tumorigenesis resulting from neonatal thymectomy in mice.

Major Findings: Long-term observations on the final fate of the persistent hyperplastic changes in the vaginal epithelium induced by injections of 50 μg of estradiol-17β (E_2) for the first five days of postnatal life are in progress. Attention is focused on the possible promoting effect of the secondary estrogen treatment as well as on the initial action of estrogen given neonatally. The oldest mice are now more than ten months of age. At present, 159 females exposed to estrogen and 187 control females are under investigation.

A study of estrogen given postpubertally is in progress in order to examine whether a secondary estrogen treatment can cause irreversible proliferation of the vaginal epithelium of mice primed neonatally with a low dose of estrogen (20 μg of E_2 for the first three days of life, which is not enough to produce ovarian-independent vaginal alterations). As part of this investigation, electron microscopic ultrastructural studies have been carried out. When mice were primed with a low dose of estrogen, it became apparent that two types of basal cells could be recognized in the vagina; one type did not respond to secondary estrogen treatment, and the second type showed a response to the estrogen treatment. Cells of the second type were seen in the non-estrogenized controls. The grade and severity of the hyperplastic vaginal alterations induced by the neonatal estrogen treatment may be correlated with the cell population of these types of cells.

Mice treated with a low dose of E_2 were ovariectomized and received ten injections of 100 μg of E_2 at two-week intervals from 60 days of age. The vagina of these animals will be examined histologically seven months after ovariectomy.

Studies have been started to estimate estrogen receptor levels in vaginal tissues of mice treated neonatally with estrogen. Preliminary data so far

obtained may suggest that neonatal estrogenization causes no significant changes in either nuclear or cytoplasmic receptor levels. However, more detailed experiments are needed in order to draw a firm conclusion.

Hyperplastic lesions in the reproductive tract of male rats were induced by neonatal DES treatment. Although a preneoplastic nature was not noticed, a papillary epithelial growth with squamous metaplasia was noticed in the dorsal wall of the urogenital sinus in and around the seminal colliculus. Irregularity of cellular and nuclear sizes and shapes was noticed. Attempts to study the response of these hyperplastic regions to the secondary estrogen treatment are now in progress. In addition, it was found that papillary epithelial growths developed rather frequently in the bladders of the male rats receiving neonatal castration and neonatal treatment with DES or estradiol benzoate.

Significance to Biomedical Research and the Program of the Institute: The rodent models for perinatal hormone-mediated oncogenesis provide the best prospect for predicting the long-term effects in both sexes of perinatal human exposure to DES and steroid estrogens. The thymectomy/ovarian carcinogenesis phenomenon is the only experimental system for exploration of the relation of aberrant lymphoid tissue maturation and subsequent autoimmune disease and neoplasia.

Proposed Course: Continue studies on the long-term effect on vagina of neonatal exposure to estrogen in mice, including the nature of permanent vaginal alterations induced by perinatal estrogen treatment and the hyperplastic lesions in the male urogenital tract induced by neonatal estrogen treatment. In addition, studies will continue on the autoimmune ovarian dysgenesis which follows neonatal thymectomy, and efforts will be made to extend these observations to similar autoimmune lesions of other organ systems (gastric mucosa, thyroid, and coagulating glands). The neoplastic potential of hyperplastic foci of epithelial cells will be studied in these affected organs. The mechanism of tumor development from dysgenetic ovaries will be studied endocrinologically by careful investigation of changes in blood hormone levels during the latency period and ultrastructurally by defining the histogenesis of these tumors with the electron microscope.

Date Contract Initiated: March 1, 1975

Current Annual Level: $69,000

ALTON OCHSNER MEDICAL FOUNDATION (NO1 CP 65764)

Title: Study of the Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats

Contractor's Project Director: Dr. Albert Segaloff

Project Officers (NCI): Dr. D. Jane Taylor
Dr. Carl E. Smith

Objective: To investigate the possible synergism of repeated low-dose radiation and estrogens in the induction of mammary tumors as a possible guide to the risk from low-dose radiation.

Major Findings: Studies by this Contractor have already demonstrated that a single dose of x-ray delivered to one mammary chain of AxC inbred rats which had also received a continuous exogenous source of diethylstilbestrol (DES) resulted in a significant increase in mammary tumors over that resulting from the estrogen alone. The optimal dose for this synergism was 150 r to the left mammary chain. The basic effort in this protocol was to duplicate this type of experiment and to compare animals given the 150 r in a single dose to animals given the radiation over an extended period of time in much smaller doses. Most of the animals have already been entered onto these protocols, but they have not been on long enough to start developing cancers.

In view of the fact that obesity is a factor in the development and prognosis of human breast cancer, and that the human mammary gland contains much fat and animal mammary glands do not, studies are also underway in animals made obese by being fed a highly palatable diet and restricted in their activity. The first animals have already been started on this phase of the study and their weight curves are now separating.

Significance to Biomedical Research and the Program of the Institute: The question of the possible carcinogenic effect of low-dose radiation has not been adequately investigated because of the very large number of animals that would be required for significant findings. The synergism with estrogen in the AxC rat, therefore, presents an ideal means of testing low-dose radiation. This is particularly important because of the possible danger from repeated mammograms over many years. Diet and obesity are increasingly being recognized as factors in carcinogenesis which must be investigated.

Proposed Course: Continue following all present studies; continue intermittent low-dose radiation on the appropriate animals; continue all observations in the animals; and develop additional protocols based on early studies in cooperation with the project officer.

Date Contract Initiated: September 30, 1976

Current Annual Level: $142,652

ALTON OCHSNER MEDICAL FOUNDATION (NO1-CP-43229)

Title: Carcinogenesis by Radiation plus Estrogen

Contractor's Project Director: Dr. Albert Segaloff

Project Officers (NCI): Dr. D. Jane Taylor
Dr. Carl E. Smith

Objective: To investigate interaction or synergism of radiation and hormones in the induction of mammary tumors.

Major Findings: The Contractor has been able to show that a single dose of x-ray delivered to one mammary chain of AxC inbred rats which had also received a continuous exogenous source of diethylstilbestrol (DES) resulted in a significant increase in mammary tumors over that resulting from the estrogen alone. None of the nonestrogen-treated control animals developed cancers. Those which were irradiated alone developed cancers at a much later time and many more benign tumors. There is a dose response relation between radiation in the range used (50, 150, or 450 r) and tumor induction in the DES-treated rats. The greatest synergism was attained with 150 r. Although in intact female AxC rats continuous administration of progesterone will inhibit both estrogen-induced mammary cancer and radiation synergism (800 r), it does not inhibit estrogen-induced mammary cancers in the absence of the ovaries. In intact female AxC rats mammary cancers induced by DES generally show regression after oophorectomy, even though the DES pellets are still in place.

Studies on the effect of early pregnancy, thus far, (all tumors not yet sectioned) indicate that early pregnancy confers protection in the number of tumors induced but not in the number of animals which develop tumors. Animals which were mated, but failed to become pregnant, developed a lower percentage of tumors and each animal had fewer tumors. Of the various hormonal treatments investigated during simulated gestation, only progesterone appears to reduce the percentage of animals developing tumors and the number of tumors which they develop.

When animals kept virginal until eight months of age (then implanted with the DES pellets and exposed to radiation) are compared to their randomly selected controls not permitted to mate, there appears to be a significant decrease in the number of tumors developed per chain at risk while the number of animals developing tumors remains stable. This apparent protection from the late pregnancy does not have an analogy in the epidemiologic studies since it is impossible to have a control group for the late pregnancies exposed to whatever carcinogenic influences may exist in the environment. The higher incidence of tumors in more animals, both with estrogen and the combination of estrogen and radiation in older animals, has previously been observed.

Significance to Biomedical Research and the Program of the Institute: Both irradiation and ovarian hormones, given individually in large doses, have been implicated in causing an increase in breast cancer of both laboratory animals and women. Epidemiologic evidence ha indicated that early pregnancy may confer substantial protection against later development of breast cancer. Studies with the animals having late pregnancies and their simultaneous controls being exposed to the estrogen and radiation have demonstrated a possible protection of late pregnancy which is impossible to show in the setting of human epidemiology.

The Carcinogenesis Program of the NCI has devoted efforts and funds to the study of these hormone problems, and this study is an extension of their efforts in this field.

Proposed Course: Contractor is completing the study of all the animals in the projected experiments. Most of them have already completed their life spans, tissues are being collected, statistical analysis of the studies are underway, and data are being prepared for publication.

Date Contract Initiated: Novermber 25, 1973

Current Annual Level: $93,825

AMERICAN HEALTH FOUNDATION (NO1-CP-33208)

Title: Studies in Colon Carcinogenesis

Contractor's Project Directors: Dr. John H. Weisburger
Dr. Ernst L. Wynder
Dr. Bandaru S. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Colon cancer is the neoplastic disease with the highest incidence in the United States. Epidemiological studies indicate that the occurrence of colon cancer is associated with a high dietary intake of fat and beef, and possibly with a lack of dietary fiber. The overall aim of this program is to acquire an understanding of the underlying mechanisms and eventually develop a sound, rational basis for preventive measures. Animal models and bioassay systems are developed and utilized to uncover a relationship between colon cancer and total dietary fat, hidden fat as present in meat, and fiber. The role of the intestinal bacterial flora is assessed, since this element participates in the overall metabolism of carcinogens, cocarcinogens, and dietary elements, as well as endogenous digestive secretions. Key substrates of interest are acid and netural sterols, in relation to macro and micro constituents of the diet. Modification of colon carcinogenesis through exogenous and endogenous chemicals, food additives, and diet components in general is under investigation.

Major Findings: A vitamin A-deficient diet reduced the colon tumor incidence induced in rats by intrarectal dosing with N-methyl-N'-nitro-N-methylguanidine.

Tumors were induced in the colon, mammary glands, and ear ducts of conventional and of germfree rats injected subcutaneously with 3,2'-dimethyl-4-aminobiphenyl (DMAB). This carcinogen is definitely less active in germfree rats than in conventional animals. Thus, the intestinal microflora increases the carcinogenic effect of DMAB in target organs.

In conventional rats, high fat (corn oil or lard at 20% level) intake led to an increased fecal excretion of neutral sterols and bile acids, compared with control low fat diets. The excretion of deoxycholic acid, lithocholic

acid, and 12-ketolithocholic acid was increased in rats fed high fat diets. The source of fat had no major influence on the excretory pattern of cholesterol and bile acid metabolites. 1,2-Dimethylhydrazine-treated animals excreted higher levels of fecal coprostanol, coprostanone, deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid than did controls.

In germfree and in conventional rats, deoxycholic acid, cholic acid, and chenodeoxycholic acid were colon tumor promoters, extending previous findings that taurodeoxycholic and lithocholic acid promoted colon cancer induction in conventional animals.

In contrast to these findings of promoting capability in the descending part of the large bowel in rats, a series of bile acids applied to the skin of mice together with benzo(a)pyrene in a classical test of cocarcinogenicity failed to demonstrate a cocarcinogenic effect. In fact, a number of bile acids and neutral sterols such as chenodeoxycholic acid, lithocholic acid, taurochenodeoxycholic acid, cholesterol, and deoxycholic acid, among others, exerted an inhibiting effect on the carcinogenicity of benzo(a)pyrene on mouse skin.

The β-glucuronidase activity in the cecal contents of rats fed diets containing high beef protein (40%) and high fat (20%) (D_3) or high soybean protein (40%) and high fat (20%) (D_1) was significantly higher compared with those fed diets containing normal beef protein (19%) and normal fat (6%) (D_4) or normal soybean protein (19%) and normal fat (6%) (D_2). No difference existed in mucosal (mammalian) β-glucuronidase activity of small intestine and colon among rats fed D_1, D_2, D_3, and D_4.

Surgical anastomosis in the right colon increased the tumor yield at the suture line in rats given subcutaneous azoxymethane. This model will be used for localization of tumor development and study of early preneoplastic lesions.

Specific and sensitive analytic systems were developed to study the metabolism of benzo(a)pyrene (BaP) in rats as a function of dose, with emphasis on low dosages. The fecal excretion was investigated of the parent compound and metabolites, including 3-OHBaP, 9-OHBaP, BaP-1,6-dione, BaP-3,6-dione, BaP-4,5-dihydrodiol, and BaP-7,8-dihydrodiol. The method is being adapted to the isolation of BaP from human stools.

Experiments are in progress to study the effect and metabolites of ad libitum, lifelong feeding of diets containing high protein and fat of animal (beef) and vegetable origin to two generations of rats on spontaneous tumor production in the colon and other organs.

Significance to Biomedical Research and the Program of the Institute: The program is of special significance since it is designed to provide important information on the relationship between diet, intestinal microflora, and colon carcinogenesis in realistic animal models. In parallel, supported by other funds (USPHS-NCI Grant CA-16382 from the National Large Bowel Cancer Project), research on the relationship of intestinal microflora, acid and

neutral sterols, and other key metabolites in varied human population groups on distinct nutritional regimens is underway. It is hoped that the data base thus generated in animal models and in man can significantly enhance our knowledge of the controllable etiological factors which play a role in cancer of the large bowel. The long-term goal is to provide a basis for rational prevention of a disease affecting over 100,000 individuals per year in the United States.

Proposed Course: (1) To develop and refine further animal models for colon carcinogenesis, including efforts to localize cancer development through specific injury or surgery. (2) To apply additional techniques for early cancer detection through endoscopy, occult blood tests, and specific enzyme changes. (3) To begin studies on the detection of carcinogens in urine, stools, and body fluids through application of rapid bioassay methodology. (4) To continue studies on the fecal chemistry and microflora in animals on high and low risk diets in the presence and absence of colon carcinogens and develop further indicator systems for high risk groups. (5) To investigate the mechanisms whereby bile acids and other sterols act as promoters during the process of colon carcinogenesis. (6) To study the possible metabolic activation of cholesterol to an active electrophile or similar reactive species in the colon. (7) To investigate further the effect of high and low risk diets with emphasis on fat, fiber, and micronutrient levels on carcinogenesis in the colon in germfree and conventional animals using dimethylhydrazine, azoxymethane, methylazoxymethanol, and 2',3-dimethyl-4-aminobiphenyl as carcinogens. (8) To study further the effect of neutral and acid sterols and other normal endogenous metabolites in these systems. (9) To investigate the effect of additional exogenous chemicals, food additives, and components in these systems. (10) To apply sensitive and specific analytical techniques to detect benzo(a)pyrene and metabolites in stools of animals and man consuming charcoal-broiled meats containing specific amounts of this compound.

Date Contract Initiated: June 30, 1971

Current Annual Level: $380,000

AMERICAN TYPE CULTURE COLLECTION (NO1-CP-65751)

Title: Development of Cell Strains from Ductal and Exocrine Portions of the Pancreas

Contractor's Project Director: Dr. Robert J. Hay

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To establish reproducible techniques for the isolation and maintenance or cultivation of pancreatic duct and exocrine cells. Such cultures will then be used for studies on chemical carcinogenesis.

Major Findings: Experiments were performed to identify factors which influence pancreatic exocrine cells maintained as colonial aggregates in vitro. Conditioned media from 14 primary and established cell lines were tested for their ability to improve maintenance or stimulate proliferation of cultured pancreatic epithelia. Interestingly, seven of these were found to stimulate proliferation in short-term experiments.

A screening program was initiated to identify histological, histochemical, or histoenzymological markers for guinea pig pancreatic duct cells. Four tests appear to be specially useful for differentiating between duct and other pancreatic epithelia. These include the PAS reaction, methyl green/pyronin Y staining plus tests for alkaline phosphatase and ATPase.

Significance to Biomedical Research and the Program of the Institute: The development of model systems for the cultivation of specific pancreatic duct and exocrine cells would provide powerful tools for studies of carcinogenesis. Definition of the factors that promote continued proliferation and function in such systems would also contribute to the basic understanding of pancreatic epithelial cell physiology.

Proposed Course: Further definition of the effects of the more active conditioned media preparations will now be attempted. Time course experiments and fractionation work with the active fluids will be initiated. Tests of known utility for identifying the various pancreatic epithelia will be applied to monitor cell separation techniques. Isopycnic and isokinetic gradient systems will be employed for the latter purpose. The progress of specific cell types in culture can also be followed using the pertinent markers.

Date Contract Initiated: February 1, 1976

Current Annual Level: $83,547

BAYLOR COLLEGE OF MEDICINE (NO1-CP-65837)

Title: Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract

Contractor's Project Director: Dr. S. Donald Greenberg

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The development of squamous cell carcinoma of the lung is a stepwise process following from the initial change of squamous metaplasia through subsequent progressive steps of mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma-in-situ and, finally, invasive carcinoma. The first objective of this project is to utilize scanning microscope photometry to accumulate a normal computer data bank of normal sputa cytology. The second goal of this project is the recognition by the computer of premalignant or atypical changes in squamous carcinoma. This later objective will be accomplished over the second and third years of the project.

Major Findings: The initial five months of this study have been utilized in developing satisfactory and reproducible techniques of sputum induction, collection, preparation and staining, and determining proper wavelengths

of maximum absorption for scanning microscope photometry of normal and abnormal cells. The wavelengths of 530 nm and 590 nm have been selected for maximum optical density of both nuclei and cytoplasm.

Significance to Biomedical Research and the Program of the Institute: The basis of this study is the need to prove morphometrically the apparent progression of advancing degrees of bronchial atypias from squamous cell metaplasia, through dysplasia to carcinoma in-situ and invasive carcinoma.

Proposed Course: Studies during the next six months will concentrate on obtaining sufficiently large populations of normal, metaplastic, midly dysplastic, moderately dysplastic, severely dysplastic, and malignant squamous cells at both 530 nm and 590 nm to conduct a complete mathematical analysis. These data will furnish the initial "training sets" to establish the most significant data descriptors to use in classification procedures.

Date Contract Initiated: September 30, 1976

Current Annual Level: $111,444

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP-55640)

Title: Criteria for In Vitro Cell Transformation by Viruses and Chemical Carcinogens

Contractor's Project Director: Dr. Robert C. Y. Ting

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: To establish criteria for in vitro carcinogenesis which will serve as in vitro endpoints that correlate with tumorigenic potential.

Major Findings: A cell aggregation assay has been developed which could provide a means of assessing the transformed status of a cell population. Over 50 normal and transformed fibroblastic and epithelial cell lines from a variety of species have been tested with this assay. Cell survival in the aggregate form over a 4-5 day period was assessed by counting the number of viable cells (trypan blue excluding) obtained by trypsinizing cell agregates into a single cell suspension. It was found that normal cell populations in the aggregate form underwent a rapid decline in the number of viable cells over a 4-5 day period, whereas transformed cell populations in the aggregate form exhibited an ability to survive and, in many cases, to proliferate. This survival ability of transformed cells in the aggregate form was found to correlate with colony formation in soft agar and with tumorigenicity in nude mice and syngeneic hosts. In addition, the aggregation assay was found to be more reliable than colony formation in soft agar for certain cell types. Also, the degree of cell proliferation in the aggregate form was found to correlate with the degree of tumorigenicity. The aggregation system can also be used in a selective manner to rapidly increase the number

of transformed cells in a population. This aggregation assay may provide a reliable means of determining the tumorigenic potential of a cell line.

Signficance to Biomedical Research and the Program of the Institute: In vitro indices of cell transformation that can successfully predict tumorigenicity would be of great value in screening programs for potential carcinogens. Such criteria would also provide a means of monitoring in vitro transformation of human cells.

Proposed Course: The major emphasis will be the establishment of criteria for in vitro transformation of human fibroblastic and rodent epithelial cells by viral and chemical carcinogens. Collaborative efforts have been initiated and will continue in order to include a greater variety of normal and transformed cell types. Also, studies will be continued for establishing correlations between tumorigenicity in nude mice and the cell aggregation criteria.

Date Contract Initiated: October 15, 1974

Current Annual Level: $135,212

CALIFORNIA, UNIVERSITY OF (at Los Angeles) (NO1-CP-33380)

Title: Fecal Flora Studies

Contractor's Project Director: Dr. Sydney M. Finegold

Project Officer (NCI): Dr. Carl E. Smith

Objective: The objective of these studies is to detect any differences in fecal microflora between subjects with colon cancer or a high risk (HR) of developing it and those without colon cancer or with a low risk (LR) of developing it.

Major Findings: A number of studies have been carried out on populations at differing risks for developing colon cancer. The first project compared two groups of 20 subjects each of Japanese ancestry, one of which consumed a primarily western diet (HR) and the other a primarily traditional diet (LR). The second project compared 25 subject with multiple adenomatous colonic polyps (HR) with 25 matched controls with negative colonoscopy (LR). A third study, compared two Seventh Day Adventist populations, one strict vegetarians (13 subjects) and the other moderate meat eaters (14 subjects). In addition, a larger number of subjects was studied only for the presence of the Clostridium paraputrificum group of organisms (this group is thought by British workers to be a key group in terms of carcinogen production). Three populations were compared - vegetarian Seventh Day Adventists (49 subjects), non-vegetarian Adventists (45 subjects), and non-Adventists on a conventional American diet (31 subjects).

The current study is comparing the fecal flora of patients with early non-obstructing colon cancer with that of matched controls. It also includes studies to determine the ability of organisms in the Clostridium paraputrificum group to produce carcinogenic chemicals from bile acids or other substances present in the intestine. Additionally, other organisms are being studied for their ability to reverse this kind of reaction. These will be done both by chemical analysis and in animal test systems.

In neither the Japanese-Western diet study nor the polyp study were there significant differences between HR and LR groups with regard to numbers of Clostridium paraputrificum or related organisms. In the adventist study, organisms in this group were found more frequently and in higher numbers in non-Adventist subjects than they were in Adventist vegetarians and non-vegetarians. Data from the three studies showed a significant differences between high and low risk populations with 21 organisms or groups of organisms. Sixteen of these were obligate anaerobes.

The studies continue to provide valuable information on the normal human fecal flora and on optimum media and techniques for defining this flora accurately and expeditiously. A major advance has been the development of a cycloserine mannose selective medium which facilitates rapid recovery and identification of Clostridium butyricum and Clostridium paraputrificum. Rifampin blood agar has been similarly useful for recovery of other members of the C. paraputrificum group.

Studies have shown that storage of fecal specimens under aerobic conditions at either room or refrigerator temperature, for up to eight hours, does not interfere with quantitative recovery of members of the C. paraputrificum group. This will facilitate future studies greatly. Studies to determine the ability of organisms in the Clostridium paraputrificum group to produce carcinogenic substances have not confirmed the results of the British workers and suggest a need for a more critical examination of the reactions that take place when these organisms are grown in the presence of bile acids or other substances present in the intestine.

Significance to Biomedical Research and the Program of the Institute: To date, a great deal has been learned of the nature of the normal human fecal flora and of optimum ways of defining it. This information will be useful in many ways other than its possible relationship to colon (and breast) cancer.

A number of differences in fecal flora composition between high and low risk groups has been noted. Differences which might be important seem to be restricted to 21 organisms or groups of organisms. Among these 21 organisms or groups may be certain key organisms which predispose to or protect against cancer.

Proposed Course: Efforts to improve and simplify bacteriologic techniques for study of bowel flora will be continued. Studies of mutagenesis and of blockade of mutagenesis by "protective" organisms will be carried out in an

in vivo system using the limited number of bacteria whose presence correlates well with risk factors. Future protocols will be designed to concentrate on a limited number of organisms of potential interest so as to permit study of larger numbers of people.

Date Contract Initiated: June 1, 1973

Current Annual Level: $235,597

CHICAGO, UNIVERSITY OF (NO1-CP-65777)

Title: Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract

Contractor's Project Director: Dr. George L. Wied

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: It is the aim of this contract to determine objective criteria on cytologic material from the respiratory tract which can be used for separating various carcinogen-induced premalignant changes from benign, toxic-induced cellular abnormalities. The study will provide long-range assessments of the biologic behavior and significance of the observed changes since the animal model in use allows repeated cytologic sampling and, therefore, follow-up of carcinogen-induced lesions for the lifetime of the animal. With the computerized cell scannings using the TICAS programs, one will be able to provide highly selective, reproducible, measurable, and thus, objective criteria. These might be visible cellular alterations which are now the important ones for the experimental and clinical cytologist, as well as the sub-visible criteria which may also be useful in an objective assessment of the status of preneoplastic lesions and in the determination of the prognosis of the lung lesion.

The evaluation of data will be conducted in such a manner that the process of carcinogenesis in the animal model can be quantitatively described, defined by objective parameters as "markers," modeled, and understood.

Major Findings: The application of the TICAS programs for human vaginal cells for automated cell and nucleus-finding routines to the material from the present animal study showed satisfactory results in the distinction between several cell changes as induced by formaldehyde, vitamin A deficiency, N-nitroso-N-methylurea, benzopyrene and ferric oxide.

It is evident, however, that the standard routines for the finding of the nuclei by computer programs need to be adapted for the specific material on hand. It can be expected that adaptive modifications will be satisfactory.

Significance to Biomedical Research and the Program of the Institute: Objective cell image analysis establishes the quantitative criteria to define the premalignant changes in the epithelial tissue of the lung.

These data can be used to fit predictive models and to enable measurements and understanding of the processes underlying carcinogenesis.

Proposed Course: The chronological milestones of the proposed project are as follows: Data acquisition from cells from the animal experiment by means of our scanning microscopes will be performed during the months 1 to 24. Statistical analyses and data reduction will be performed from month 4 to month 34. The systems training (supervised learning procedure) will be accomplished from months 12 to 24, followed by marker definition from months 24 to 30. The nonsupervised learning procedure will be done during the months 28 to 34, as well as the development of the trend descriptors, while the final two months of the project (months 34-36) will be devoted to the compilation of the final report.

Date Contract Initiated: September 30, 1976

Current Annual Level: $154,222

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CP-55641)

Title: Fibrinolysis as a Parameter of In Vitro Transformation

Contractor's Project Director: Dr. William F. Benedict

Project Officer (NCI): Dr. Stuart H. Yuspa

Objective: The main objective of this contract is to determine the significance of increased fibrinolytic activity as related to the expression of malignancy in vivo as well as malignant transformation in vitro. A corollary of this objective is to determine how rapidly after malignant transformation in vitro an increase in fibrinolytic activity occurs.

Major Findings: Over the last year studies have continued to look at the role of increased plasminogen activator as related to the expression of malignant transformation in vitro. It was found that increases in plasminogen activator are correlated with chemical transformation of the C3H/10T½CL8 mouse cell line. In collaboration with Drs. Carl Barrett and Paul Ts'o at Johns Hopkins University, it was also shown that an increase in plasminogen activator production occurs in the second to third passage after transformation of hamster cell by benzo(a)pyrene. Furthermore, Dr. Laug, working in Dr. Manfred Rajewsky's laboratory utilizing the in vivo/in vitro transformation system of rat brain cells has found that untransformed Stage I cells have no increase of plasminogen activator, whereas tumorigenic Stage IV cells have a marked increase in plasminogen activator. These studies indicate that an increase in plasminogen activator could be an early marker for transformation. It has also been found that all of the human neuroblastoma cell lines which have been developed and studied here have high levels of plasminogen activator.

Significance to Biomedical Research and the Program of the Institute: Increased plasminogen activator production may be important to the expression of tumorigenicity as well as an early marker for oncogenic transformation in vitro. These findings may be particularly relevant to future studies, especially since human transformation systems are now being developed. Changes in the levels of plasminogen activator may prove to be an important marker for the transformed state of human cells as has been shown in other mammalian cells.

Proposed Course: The relationship of increase in plasminogen activator concentration to transformation of various mammalian cells will continue to be studied, including cells of epithelial and of human origin. The mechanism of the induction of plasminogen activator as well as its subcellular localization will continue to be investigated.

Date Contract Initiated: October 21, 1974

Current Annual Level: $40,000

COLORADO, UNIVERSITY OF (NO1-CP-75875) [Formerly - Stanford University (NO1-CP-55649)]

Title: Tissue Interactions in Induction and Perpetuation of Hormonally-Induced Permanent Cellular Alterations

Contractor's Project Director: Dr. Gerald R. Cunha

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: To determine the role of tissue interactions in normal, abnormal, and neoplastic growth and differentiation of the vagina, paying particular attention to the role of stroma in expression of epithelial differentiation, and to investigate the mechanism of cell-cell interactions in normal and abnormal development of the urogenital tract.

Major Findings: On the basis of earlier studies which demonstrated (1) that induction and perpetuation of ovary-independent persistent vaginal cornification occurs via an interaction between epithelium (E) and stroma (S) in which stroma plays an inductive role, and (2) that in normal vaginal and uterine development the stroma induces and specifies morphogenesis and cytodifferentiation, long-term studies have been initiated employing recombinants composed of untreated and neonatally estrogen-altered tissues to determine directly the role of tissue interactions in estrogen-induced vaginal carcinogenesis.

Ultrastructural studies have provided data which suggest that the tissue interactions which occur during neonatal vaginal differentiation are matrix interactions since in all cases the E + S are separated by a continuous

basal lamina. Evidence also demonstrates that the E actively engulfs basal laminar material via a microphagic process which may be the mechanism of information transfer from the inductive S to the responding E. In addition, the well-documented process of epithelial cell selection induced by estradiol in neonatal mice, appears to be mediated by differences in the adhesion of certain vaginal epithelial cells to the basal lamina. In E-treated vaginae electron-dense basal cells appear and develop approximately four times the number of hemidesmosomes found in neighboring light cells which are apparently displaced from the basal lamina by the electron-dense cells.

Significance to Biomedical Research and the Program of the Institute: The normal morphogenesis and cytodifferentiation of many embryonic structures has been shown to be dependent on an interaction between epithelium and mesenchyme. In addition, maintenance of adult structure and function also appears dependent on such an interaction, and some investigators have suggested that carcinogenesis may occur as a result of an alteration of the normal interaction between epithelium and stroma. This hypothesis, for technical reasons, has not been confirmed. However, use of the vagina as a model system provides a technically-feasible approach to this fundamental question.

In addition, this research deals with the broader question of how intercellular communication occurs in various morphogenetic tissue interactions in embryonic, neonatal, and adult organs. It is believed that understanding of normal processes of morphogenesis and cytodifferentiation will provide the necessary background to permit formulation of protocols which will directly determine the relationship between perturbations of the normal process of cytodifferentiation and the development of carcinoma.

Proposed Course: Recombinations of epithelium and stroma from vaginas of estrogenized and untreated female mice will be repeated and the grafts allowed to grow in ovariectomized hosts for 12 to 18 months to determine if stromal induction of epithelial hyperplasia and parakeratosis will lead to epidermoid carcinoma of the untreated vaginal epithelium. In addition, an extensive series of heterotypic recombinations between epithelium and stroma from various regions of the reproductive, integumentary, and digestive systems will be constructed in order to determine the roles of epithelium and stroma in the normal pre- and postnatal development of the reproductive tract.

Studies on the mechanism of vaginal epithelial cell selection via differential cellular adhesion (hemidesmosomes) and the mechanism of tissue interactions via epithelial uptake of the extracellular matrix will continue through employment of ultrastructural, autoradiographic, histochemical, and immunocytochemical approaches, coupled with the tryptic-separation and recombination technique.

Date Contract Initiated: December 15, 1976

Current Annual Level: $133,688

COLORADO, UNIVERSITY OF (NO1-CP-65849)

Title: In Vitro Cultivation of Normal, Epithelial, Human Prostatic Cells

Contractor's Project Director: Dr. Mukta M. Webber

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: The major objective of this project is to develop an in vitro model which could be used to investigate early steps in human prostatic carcinogenesis. In order to develop this model, procedures must be developed for the cultivation of normal prostatic epithelium of human origin in pure cultures. The immediate objectives are to isolate normal human prostatic epithelium; to establish nutrient requirements for its growth and maintenance in vitro, and to characterize the epithelium grown in vitro to establish its normal, human prostatic origin.

Major Findings: Procedures for securing normal human prostatic tissue from organ transplant donors have been established. These donors are the best source of viable, normal tissue. Autopsy specimens as a source of normal prostatic tissue for explant cultures were originally found to be less than satisfactory. However, a new procedure has been developed for isolating prostatic glands, which has given success in growing prostatic epithelium from such specimens. Procedures are being established which will provide optimum conditions for securing viable tissue from autopsies, thus making these also a good source of tissue.

Methods for the separation of prostatic glands from the stroma using collagenase have been successfully developed. Vigorously growing, pure cultures of prostatic epithelium have been established from the isolated glands. Six different media (BME, CMRL, MEM, F-12, RPMI and 199) have been tested for their effects on the growth of prostatic epithelium and the medium inducing the optimum growth has been selected. Effects of glutamine on the growth of prostatic epithelium have been determined. Addition of L-glutamine at 1 mM level to freshly prepared medium enhances the growth of these cells. Effects of different sera on the growth of prostatic epithelium using explant cultures have been investigated, and the serum combination inducing the optimum growth has been determined.

Effects of insulin on the growth of prostatic epithelium in vitro have been studied. Concentration of insulin required to induce the optimum growth of prostatic epithelium in culture has been established. Insulin requirements of normal prostatic epithelium in vitro were found to increase with the age of the donor. Insulin not only induces a marked increase in the growth of prostatic epithelium but it also increase the lifespan of these cells in culture. Effects of insulin on the ultrastructure of cultured prostatic epithelium were examined. Increase in the number of pinocytotic vesicles and in the size and number of mitochondria is related to the increased uptake of nutrients from the medium and an increase in the cellular metabolism. Selective, growth-enhancing influence of insulin in favor of prostatic epithelium and inhibition of fibroblasts has been established.

Although isolation of prostatic epithelium has been accomplished using collagenase, its long-term maintenance still remains to be resolved. Explant cultures were found to have a longer lifespan than those initiated from isolated glands. Insulin does increase the lifespan of both types of cultures; however, gland cultures do not survive as long as the explant cultures. On the basis of the preliminary studies, the possible role of stroma in the maintenance of prostatic epithelium is suspected.

Studies on the characterization of prostatic epithelium using ultrastructure and prostatic acid phosphatase are being continued. Desmosomes, a characteristic organelle of epithelial cells, have been consistently found in the prostatic epithelial cultures.

Significance to Biomedical Research and the Program of the Institute: Availability of an in vitro model will make it possible to conduct studies on the early steps in prostate carcinogenesis: the target cell-carcinogen interaction; the role of multiple agents and co-carcinogens in transformation and the mechanism of induction and selection. An in vitro model could also be used 1) for testing chemotherapeutic agents; 2) as antigenic targets for monitoring cell-mediated anti-tumor reactivity in studies on immunotherapy; 3) as a cell system for viral vaccine production; 4) for screening drugs, food additives, and environmental carcinogens and forecasting their effects; 5) for studies on cell nutrition, metabolism, growth, differentiation, and aging; and 6) for studies on the role of cell aging in prostatic carcinogenesis.

Proposed Course: Studies on growth enhancement and maintenance will continue using a variety of agents. Effects of these agents on the surface architecture and ultrastructure will be investigated to determine the well-being of the cells in the culture environment provided. Characterization studies of cells grown in vitro to establish their prostatic and normal origin will be conducted. Studies on establishing the normal origin of cells grown in vitro, using several in vitro tests for transformation, are being initiated. A new, rapid and quantitative method for measuring growth of cells in vitro is being developed.

Date Contract Initiated: September 30, 1976

Current Annual Level: $125,832

DARTMOUTH COLLEGE (NO1-CP-65776)

Title: Development of Animal Model for Oat Cell Carcinoma of the Lung

Contractor's Project Director: Dr. George D. Sorenson

Project Officer (NCI): Dr. Michael B. Sporn

Objective: The objective of the project is to develop a satisfactory animal model of oat cell carcinoma of the human lung either through xenogeneic transplantation of human oat cell carcinoma cells from established cell lines or through primary induction of an analagous type of primary animal tumor.

Major Findings: Since this is a recently awarded contract, there are no major findings to report at this time. However, tumors have been established thus far in nude mice using cell lines from three small cell carcinomas of human lung.

Significance to Biomedical Research and the Program of the Institute: The successful development of one or more animal models of human oat cell carcinoma could be expected to contribute significantly with respect to supplying information about the pathobiology of this important type of neoplastic disease. In addition, such a model would provide a much needed in vivo system for testing the usefulness of various therapeutic approaches to the control of the tumor.

Proposed Course: The approach is twofold: (1) Present efforts to transplant additional tumor xenografts into nude and/or immunologically depressed mice will be continued with an emphasis on identifying those models most appropriate for evaluating selected investigative parameters; (2) Primary chemical induction of pulmonary carcinoma in weanling rats will be initiated. Selected carcinogens are being tested which are known to be correlated with a high incidence of lung tumor in man and which may be expected to metabolically localize in what are presumed to be the cells of origin for human oat cell carcinoma.

Date Contract Initiated: September 30, 1976

Current Annual Level: $171,469

DARTMOUTH COLLEGE (NO1-CP-55708)

Title: Chemical and Structural Requirements for Pancreas Uptake and Excretion: Rats and Hamsters

Contractor's Project Director: Dr. Thomas J. Curphey

Project Officer (NCI): Dr. Carl E. Smith

Objective: To determine the chemical and structural properties of compounds having a propensity for pancreas uptake and excretion through the pancreatic duct.

Major Findings: The distribution of fifty compounds into plasma, pancreas, liver, and kidney of rats and hamsters has been measured, using radiochemical tracer techniques, and evidence for excretion into the pancreatic duct system has been sought. There has been active uptake of a variety of alpha amino acids by the pancreas. The pancreas appears to show a low degree of structural selectivity in uptake of alpha amino acids, provided both free amino and carboxyl groups are present. Acylation of the amino group or esterification of the carboxyl group abolishes active uptake, while alkylation on nitrogen has little effect. Preliminary data suggest that

optical configuration at the alpha carbon atom may not be a significant structural requirement for uptake of radioactive alanine, both D and L isomers being taken up to a similar extent. Studies with labeled polycyclic aromatic hydrocarbons have shown an initial rapid uptake of radioactivity by the pancreas, followed by a slower wash out.

Significance to Biomedical Research and the Program of the Institute: The goal of this project is to define the structural determinants for localization of chemicals in the pancreas as a result of absorption and distribution through the blood stream, and further to determine which types of compounds might be excreted into the duct system. Such knowledge is sought to form a basis for understanding which, if any, environmental chemical carcinogens are to be most suspect as possible causes of carcinoma of the pancreas. Identification of such chemicals would form a basis for decreasing exposure and risk in humans. A further benefit of defining the structural determinants for pancreatic localization is that it would permit rational design of pancreas-specific carcinogens (for model studies), chemotherapeutic agents, and organ-visualizing agents.

Proposed Course: Further studies will be made of localization of amino acids and related derivatives, known pancreatic carcinogens, and selected polycyclic aromatic and heteroaromatic compounds.

Date Contract Initiated: June 30, 1975

Current Annual Level: $117,464

DARTMOUTH COLLEGE (NO1-CP-33378)

Title: Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The principal goal is to establish an animal model for induction of pancreatic adenocarcinoma in rats using chemicals with two characteristics: (1) compounds which concentrate in the normal pancreas, and (2) compounds which appear to be potential carcinogens on the basis of chemical structure, mutagenic activity in bacteria, and/or ability to cause malignant transformation of mammalian cells in culture. To achieve these characteristics several new nitroso derivatives of amino acids have been synthesized.

Major Findings: Factors influencing the development of pancreatic adenocarcinoma in azaserine-treated rats have been examined. The effectiveness of azaserine for induction of atypical acinar cell nodules (AACN) in several different species and rat strains has been evaluated in studies of four or six months duration. Weanling rats develop more nodules than adult rats following equivalent regimens, and male rats are more responsive than female rats. About 10% of Wistar (random bred) rats are relatively insensitive

to nodule induction by azaserine while Wistar/Lewis (inbred) rats have been uniformly responsive, and Fischer (inbred) rats have been uniformly insensitive. One mouse strain was sensitive while strain 13 guinea pigs and one hamster strain were insensitive. In longer term studies of azaserine carcinogenesis, oral administration was as effective as intraperitoneal injection, and the incidence of pancreatic cancer was twice as great in males as in females.

Preliminary studies have been completed with three methylnitrosourea amino acids, N^{ε}-(N-methyl-N-nitrosocarbamoyl)-L-lysine (MNCL), N^{δ}-(N-methyl-N-nitrosocarbamoyl)-L-ornithine (MNCO), and N^{γ}-(N-methyl-N-nitrosocarbamoyl)-L-diaminobutyric acid (MNDABA). These compounds are carcinogenic in rats and induce renal, skin, and breast tumors within six months after first treatment. Most renal neoplasms are malignant mixed tumors although small tubular adenomas are also seen. The skin lesions encompass a broad spectrum including apparently benign epidermal inclusion cysts as well as basal cell carcinoma, adnexal carcinoma, and squamous carcinoma. The breast tumors include adenocarcinomas and fibroadenomas. Qualitatively, the lesions induced by these three agents are similar. All three have striking effects in the pancreas including the induction of AACN like those induced by azaserine. Higher doses also induce fibrotic areas with epithelial lined cysts. MNDABA is most effective, and MNCL is least effective in inducing pancreatic AACN. MNDABA-treated rats have developed extremely dysplastic foci of acinar cells which appear malignant histologically. There was no evidence of vascular invasion or metastases. Single doses of three mM/kg of MNCO have produced focal necrosis in the pancreas without lethality. The similarity of acinar cell abnormalities induced by methylnitrosourea amino acids to those induced by azaserine and 4-hydroxyaminoquinoline-1-oxide suggests that these compounds will induce adenocarcinomas in studies of 1-2 years duration.

Significance to Biomedical Research and the Program of the Institute: This approach has yielded a biological model of pancreatic carcinogenesis in which cancers appear to arise in acinar cells. Comparison of results with several agents should help elucidate the importance of pancreatic localization and chemical structure as determinants of pancreatic carcinogenesis by chemicals. Such information will form one basis for recognizing potential pancreatic carcinogens in the environment.

Proposed Course: Evaluation in vivo of methylnitrosourea amino acids as experimental pancreatic carcinogens will continue. Factors affecting the induction of pancreatic cancers by azaserine and methylnitrosourea amino acids, e.g., rat strain, age, route and duration of treatment, and effect of possible cocarcinogens will be studied further.

Date Contract Initiated: June 28, 1972

Current Annual Level: $86,252

ERDA-NCI INTERAGENCY AGREEMENT (BROOKHAVEN NATIONAL LABORATORY) (Y01-CP-60219)

Title: Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: To investigate the mammary adenocarcinoma response in AxC female rats treated with diethylstilbestrol (DES) when the x-ray dose is given either singly or fractionated into 4 or 8 fractions, at 3 different x-ray doses.

Major Findings: This is a new contract. As yet no major findings can be reported. Female AxC rats are being bred and aged so they can be entered into the study.

Significance to Biomedical Research and the Program of the Institute: It has been reported that exposure to ionizing radiation is associated with an increased risk of developing breast cancer in the human female. There is little agreement whether or not successive doses of x-irradiation would be expected to be additive. A portion of women who receive repeated radiation exposures may also be receiving sex steroids. The current project will use estrogenized female rats of the AxC strain as a test system to evaluate the effectiveness of divided doses of x-rays on mammary carcinogenesis.

Proposed Course: As rats reach the age of 6 months they will be irradiated with a total dose of 80 rad, or 40 rad or 20 rad, either in a single fraction, or 4 fractions 2 months apart, or 8 fractions one month apart. After the radiation regimen has been completed DES will be administered and the rats studied for mammary carcinogenesis.

Date Contract Initiated: September 30, 1976

Current Annual Level: $190,792

ERDA-NCI INTERAGENCY AGREEMENT (BROOKHAVEN NATIONAL LABORATORY) (Y01-CP-30213)

Title: Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: To determine if the synergistic interaction between x-irradiation and estrogen on mammary gland carcinogenesis in the female AxC rat can also be observed for neutron radiation; and to understand the mechanism of such synergism.

Major Findings: A synergistic interaction between diethylstilbestrol (DES) and x-irradiation on mammary adenocarcinoma formation in AxC female rats has been extended to DES and neutron radiation in AxC female rats, but this synergism was not detected in Sprague-Dawley female rats. DES has been found to induce much higher levels of serum prolactin in AxC rats than in Sprague-Dawley rats. The interaction between DES and the chemical carcinogen dimethylbenzanthracene on mammary adenocarcinoma formation in AxC female rats appears to be more nearly additive than synergistic. The relative biological effectiveness (RBE) of neutrons as compared to x-rays for synergism with DES in AxC female rats on mammary adenocarcinoma formation is under study. Iterminresults suggest an RBE value of about 10 at large neutron doses and about 50 at low neutron doses.

Significance to Biomedical Research and the Program of the Institute: Because it has been reported that women exposed to repeated fluoroscopic examinations, women survivors of atomic bombings and possibly women treated with x-ray for acute postpartum mastitis all have an increased risk of developing breast cancer, radiation exposure is now associated with breast cancer of the human female. With more frequent use of neutron services in industry, research, and medicine, the possibility of increased exposure of women to neutron-irradiation is expanded. Also, the use of steroids for contraceptive and/or replacement therapy has increased. Thus, the interactions of neutron-irradiation and sex steroids in regard to mammary carcinogenesis should be evaluated. This project is an attempt to develop an animal model, and to understand the model itself, so that the qualitative aspects of mammary carcinogenesis in man may be evaluated in terms of cause and prevention of breast cancer.

Proposed Course: To finish experiments in progress and to learn if dividing the neutron dose into 2 equal fractions reduces the effectiveness of neutron radiation interaction with DES.

Date Contract Initiated: June 28, 1973

Current Annual Level: 118,970

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. (NO1-CP-65763)

Title: Resource for Microscopic and Autoradiographic Technology

Contractor's Project Director: Dr. Beverly Y. Cockrell

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: Contractor serves as a resource for preparation and examination of tissues both for high resolution autoradiography (1 micron sections of plastic-embedded tissues) and for high resolution light and electron microscopy. Microscopy is used to provide data on (1) the pathogenesis of tumors of various target organs such as lung, colon, skin, pancreas, and prostate; and (2) the localization of labeled compounds, including carcinogens, into cellular organelles by autoradiographic techniques.

Tasks, during the course of the NCI's collaborative research program, are identified for performance under this contract. Such tasks are either (1) a professional, collaborative relationship in which the contractor participated in the design, execution and evaluation of the task as well as co-authorship of any resulting publications, or (2) performance of an NCI protocol under direction of an NCI project representative. Each task is a relatively short study of less than six months duration.

Major Findings: This Contract has only recently been awarded; however, two tasks have already been initiated. These two projects are: (1) "Improvement of Organ Culture Conditions of Human and Nonhuman Epithelial Tissue Cell Systems" and (2) "Sulfaguanidine Protection of Rat Liver from the Effects of Carbon Tetrachloride." It is too early for results to be available.

Significance to Biomedical Research and the Program of the Institute: Chemical carcinogens are becoming increasingly widespread throughout man's environment. In order to protect a human from the carcinogenic effects and to decrease his susceptibility to such chemicals, data must be gathered concerning the mode of action of the carcinogens in various organs, the susceptibility states of various tissues, and the development of models for the study of specific organ carcinogenesis.

This project is most signficant to the Collaborative Program of the NCI. Within the Experimental Pathology Branch, an integral part of this NCI Program, the need exists to extend and supplement the microscopic and autoradiographic techniques which are performed in research. No other way currently exists for such essential studies to be performed, without which the progress of the NCI would be seriously hampered and delayed. This resource is available to all investigators, NCI staff, and contractors in the Collaborative Carcinogenesis Program.

Proposed Course: The two projects indicated above will be completed, and further support efforts initiated as required.

Date Contract Initiated: September 23, 1976

Current Annual Level: $9,120

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-55656)

Title: Study of the Potential for Metabolic Activation in Animal Pancreas

Contractor's Project Director: Dr. Owen Black, Jr.

Project Officer: Dr. David G. Longfellow

Objective: To determine if experimental animal pancreata intrinsically possess the potential for metabolic activation or biotransformation of carcinogens and other agents.

Major Findings: The capability of the Long-Evans rat pancreas to biotransform the carcinogen 7,12-dimethylbenzanthracene (DMBA) has been examined. In vitro experiments, designed to separate hepatic from pancreatic capabilities, disclosed that pancreatic biotransformation resulted in metabolites qualitatively similar to hepatic matabolites. The capability of both liver and pancreas was less than that seen for 3-methylcholanthrene (3MC). Pretreatment of the rats with either benzo(a)pyrene (BP) or phenobarbital (PB) induced the metabolic capability in the liver but not in the pancreas.

In vivo experiments examined the relative concentration of metabolites at 2, 5, and 16 hours after injection of DMBA. Content of radioactivity in pancreas at 2 hours was similar to that in the liver. The radioactivity in the pancreas remained high for 16 hours while liver radioactivity declined nearly fivefold. The percent of water soluble metabolites in liver was high at all time periods, five to six times higher than that in the pancreas. Additional time periods will be examined.

Liver and pancreas were examined to determine whether fluorescent metabolic products were being sequestered in particular cell types and whether acinar and ductal cells had similar capabilities for biotransformation. These studies showed that acinar and ductal tissue had similar capabilities to biotransform 3MC to fluorescent products. Further, ductal epithelium was exposed to greater concentrations of carcinogens than acinar tissue. Ductal tissue exposure came from both pancreatic and hepatic sources.

Significance to Biomedical Research and the Program of the Institute: It has long been thought that biotransformation is a prerequisite for carcinogenesis for many carcinogens, including polycyclic hydrocarbons. The capacity for metabolic activation in the pancreas must be determined, and the role of such activation assessed in carcinogen-induced pancreatic carcinoma. The present observations confirm the metabolic capability for pancreatic biotransformation of DMBA as well as 3MC. The greater exposure to carcinogenic metabolites found for ductal epithelium compared to that for acinar tissue supports the morphological observations of the ductal origin of tumors produced by a number of carcinogens.

Proposed Course: The in vivo study of the metabolic activation of DMBA will be extended to other time periods. In vitro studies will be carried out with BP. Fluorescence microscopy will be used to observe the tissue response to metabolites of DMBA and BP.

Date Contract Initiated: June 30, 1975

Current Annual Level: $64,096

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-43282)

Title: Epidemiological Studies of the Incidence of Colon Cancer among Blacks and Whites

Contractor's Project Director: Dr. Warren H. Gullen

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To investigate, via a case-control study in nine hospitals, the relationship between dietary factors (especially fats, animal proteins, and fiber) and cancer of the colon.

Major Findings: None. Data collection still in process.

Significance to Biomedical Research and the Program of the Institute: The role of diet in the etiology of colon cancer is a subject of great interest and increasing scientific activity. A limited number of epidemiological studies have produced provocative but controversial findings. This study is intended to shed further light on the subject.

Proposed Course: Data collection will continue until the latter part of 1977. Analyses should be complete by May 30, 1978.

Date Contract Initiated: June 30, 1972

Current Annual Level: 0

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NO1-CP-33273)

Title: Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

Contractor's Project Director: Dr. John B. Little

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To examine some of the factors which influence the carcinogenic response to respiratory carcinogens, to develop appropriate models for studying interactions between these agents, to determine whether a synergistic or potentiating effect exists between alpha radiation and chemical carcinogens in the production of lung cancer, and to investigate conditions which may predispose to or be associated with such synergistic interactions.

Major Findings: Lung cancer has been induced in hamsters by the intratracheal instillation of either ^{210}Po or BP adsorbed onto hematite particles. No tumors occurred in untreated or hematite-treated control animals. Distribution studies with polonium either in saline or on various concentrations of hematite particles indicate that the uniform distribution of alpha-emitting radionuclides as occurs following saline instillation is equally or more carcinogenic as is the nonuniform distribution or "hot-spots" seen with the particulate instillation. Localization studies have shown that intratracheally administered ^{210}Po delivers its major dose to the epithelium of the distal airways, as does BP administered in a gelatin-saline suspension. BP administered on hematite carrier particles, however, localizes largely in the tracheal and bronchial epithelium. These are the respective regions where

the malignant tumors induced by each agent arise. Tumors have been induced in about 10% of hamsters receiving alpha radiation doses of only 15-75 rads from ^{210}Po. A significant synergistic effect has been demonstrated between low doses of ^{210}Po and BP administered sequentially; a similar synergistic interaction has been demonstrated in an in vitro transformation system. Preliminary data suggest that cell proliferation between exposures may play an important role in such interactions.

Significance to Biomedical Research and the Program of the Institute: The studies are addressed to a very critical problem in the evaluation of human lung cancer hazards which may result from the combined effects of radiation and chemical carcinogens. Synergistic relationships between alpha radiation and chemical carcinogens have been implicated in the high incidence of occupational lung cancer in man (uranium, hematite, and flurospar miners). Previous studies have shown that ^{210}Po, a component of tobacco smoke, accumulates in the bronchial wall, especially the segmental bifurcations of smokers. This is a region where a high percentage of the bronchial carcinomas are found. The possible role of ^{210}Po, acting as a cofactor along with other chemicals in tobacco smoke, needs clarification. Results from this project could have a direct bearing on the evaluation of lung cancer hazards in man and on future developments in the whole Lung Cancer Program.

Proposed Course: Studies will be continued to assess synergistic effects between BP and ^{210}Po administered simultaneously and sequentially and to evaluate the importance of cell proliferation in such interactions. The effect of dose rate on the induction of lung cancer by alpha radiation will be studied following moderate and low total doses. Contractor will continue parallel studies with an in vitro transformation system, which may allow the identification of particularly hazardous combinations of respiratory carcinogens.

Date Contract Initiated: March 30, 1971

Current Annual Level: $126,143

HAZLETON LABORATORIES, INC. (NO1-CP-23287)

Title: Induction of Adenocarcinoma of the Prostate

Contractor's Project Director: Dr. Dan W. Dalgard

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective is the induction of prostatic adenocarcinomas in the dog through direct intraprostatic inoculation of various chemical carcinogens and manipulation of the hormone system. Approximately 260 dogs of three distinct age groups (immature, mature, and aged) have received repeated carcinogen inoculations. A portion of each age group has been left intact, castrated, or given supplementary androgens. The chemical carcinogens under study include 3-methylcholanthrene (3-MC), N-methylnitrosourea (MNU), cadmium chloride (CC), and N-methylnitrosoguanidine (NG).

Major Findings: Fibrosarcomas were induced in 39% of 23 sexually immature dogs which received up to 3 inoculations of 3-MC. Induction time ranged between 8-12 months. No other compounds have yielded any tumors in any of the dogs.

The majority of the animals were recently sacrificed after 17 months in a long-term holding facility. All inoculations were discontinued and the animals were being held for an appropriate period to allow possible expression of latent tumor.

The results of the gross necropsy were essentially negative in that no adenocarcinomas were identified. Nine transitional cell carcinomas involving the prostate were detected in animals which received 3 MC at 1-2 years of age. Other tumors (4) were noted in other organs, but were not believed to be compound-induced.

Significance to Biomedical Research and the Program of the Institute: Deaths due to prostatic adenocarcinoma approach 20,000 in the U.S. annually. Unfortunately, there are few animal species which exhibit a truly comparable disease entity. The dog, however, does develop age associated prostatic hyperplasia which is quite similar to that seen in man. Morphologically, the gland is more similar than that of the rodent and most other conventional laboratory species.

Proposed Course: This contract has been completed, with the submission of a final report, on February 28, 1977.

Date Contract Initiated: June 24, 1972

Current Annual Level: 0

IIT RESEARCH INSTITUTE (NO1-CP-43289)

Title: Supply of Animals Treated with Epithelial Carcinogens

Contractor's Project Directors: Dr. Richard C. Moon
Dr. Clinton J. Grubbs

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The primary objective of this program is to supply animals with preneoplastic lesions of the trachea, urinary bladder, esophagus, and mammary gland to those organizations designated by the National Cancer Institute.

Major Findings: Initial experiments to induce respiratory lesions with benzo(a)pyrene-coated particles were not acceptable due to the lack of reproducibility of carcinogen preparation, the length of time necessary

for tumor induction, and the occurrence of tumors in sites other than the trachea. A new technique which allows induction of preneoplastic lesions in a circumscribed region of the hamster trachea has become available and is now under extensive development and evaluation. A special catheter has been fabricated which delivers the carcinogen (N-methyl-N-nitrosourea) to approximately a 6 mm length of the trachea 10 to 16 mm distal to the vocal cords. An anesthesia apparatus has also been constructed for maintaining several hamsters in different states of anesthesia for the intratracheal instillations. Catheter tips which permit the intratracheal instillation of a carcinogen in both 7 and 15-week old hamsters have also been fabricated. Animals are currently being treated once or twice each week for various lengths of time. Preneoplastic changes of the tracheal epithelium are being monitored by serial sacrifices of the animals and by aspirate cytology.

Hamsters treated for various periods of time with either 0.5 or 1% N-methyl-N-nitrosourea, as well as tracheal cytological samples from such animals, were provided to investigators designated by NCI. In addition, Contractor has supplied the NCI with mammary glands from rats treated with the carcinogens 7,12 dimethylbenz(a)anthracene and N-methyl-N-nitrosourea for their studies on mammary hyperplasia. Furthermore, two strains of rats receiving various dosages of butyl-(4-hydroxybutyl)nitrosamine for various periods of time have been supplied to various investigators designated by NCI for studies relative to urinary bladder carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: This contract will provide reproducible tumor models for the induction of preneoplastic lesions of several epithelial tissues. In this way, animals treated with carcinogens can be supplied to investigators for either in vivo or in vitro experiments.

Proposed Course: To continue experiments designed to improve existing epithelial tumor models; to determine the optimum conditions for rapidly inducing preneoplastic changes; and to continue to provide carcinogen-treated animals to investigators designated by NCI.

Date Contract Initiated: May 20, 1974

Current Annual Level: $161,665

IIT RESEARCH INSTITUTE (NO1-CP-23292)

Title: Studies of Modulating Factors in Epithelial Carcinogenesis

Contractor's Project Directors: Dr. Richard C. Moon
Dr. Clinton J. Grubbs

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The primary objective of this program is to investigate those factors which affect the susceptibility of several epithelial tissues to a carcinogenic insult and to assess the anticarcinogenicity of various retinoids (vitamin A and analogs of vitamin A) as related to epithelial tumors. The emphasis of the program is directed toward the modulation or prevention of carcinogenesis of those epithelial tissues exhibiting a high incidence of cancer in the human being. Thus, the evaluation of several retinoids in tumor models for epithelial carcinogenesis of the respiratory tract, breast, bladder, and esophagus are under investigation.

Major Findings: Mammary Carcinogenesis. Pilot studies at IITRI demonstrated that injections of N-nitrosomethylurea to Sprague-Dawley female rats resulted in a high incidence of mammary adenocarcinomas. Thus, a study was performed to determine the effect of retinyl acetate (RA) on this tumor model. Female rats received 5 mg NMU/100 g BW intravenously at 50-55 days of age followed by a second injection of 5 mg NMU/100g BW 7 days later. Eighty-three percent of the animals developed multiple mammary cancers by 175 days after the initial NMU injection. Furthermore, a good dose response relationship was observed using smaller doses of NMU. At total doses of 2.5, 5, and 10 mg NMU/100g BW, the percent incidence of mammary cancer was 20, 41, and 83%, respectively. Animals treated with the above dosages of carcinogen and receiving RA (250 mg/kg of diet) beginning 3 days after the 2nd carcinogen injection showed a percent incidence of mammary cancer of 0, 23, and 50% as compared with their respective controls of 20, 41, and 83%. Furthermore, the number of cancers was markedly reduced and the latent period for appearance of palpable cancer was prolonged. It is evident from these data and previous studies that the retinoids (natural and synthetic) suppress both NMU- and DMBA-induced mammary cancer in rats.

Bladder Carcinogenesis. Prior studies performed at IITRI indicated that butyl-(hydroxybutyl)nitrosamine (OH-BBN) would selectively induce urinary bladder cancers in rats following its intragastric administration. However, sufficient data has not yet accumulated to determine which strain or dose regimen is most suitable for inducing bladder lesions. During this contract period, a study to determine the effect of 13-cis retinoic acid on OH-BBN-induced bladder tumors in Fischer rats was completed. Histological evaluation of the bladders indicated that 13-cis retinoic acid reduces the incidence of the bladder cancer by approximately 50% and decreases the severity of the lesions. A study was also initiated to evaluate 13-cis retinoic acid, RO 10-9359, and RP 11-1430 on bladder cancer in Wistar rats. Each retinoid was evaluated at a weekly dose level of 400 mg OH-BBN for either 4 or 6 weeks (total dose 1.6 or 2.4 g). The animals have been sacrificed, and the tissues are being processed for histologic study. Additional studies to determine the effect of 13-cis retinoic acid on various stages of OH-BBN-induced bladder lesions and to develop a bladder tumor model in mice are in progress.

Esophageal Carcinogenesis. Although several of the methylalkylnitrosamines have been shown to induce carcinomas of the esophagus, the dose and route of administration of the carcinogen and the strain of animal in which the

compounds are most effective has not been determined. Male and female Wistar rats have been treated with weekly injections of 30 mg methylbutylnitrosamine/kg BW for 6 weeks beginning at 10 days of age. The animals were divided into various groups and treated with either 13-cis retinoic acid, RO 10-9359, and RO 11-1430. The rats were sacrificed at 8 months of age and the tissues are being processed for histological evaluation. An additional study to evaluate these retinoids in Fischer female rats treated with methylbenzylnitrosamine at three different total dosages (2.5 mg/kg BW weekly for 20 weeks, 1.25 mg/kg BW weekly for 20 weeks, and 2.5 mg/kg BW for 10 weeks) is in progress.

Respiratory Carcinogenesis. A specially designed intratracheal catheter for the administration of non-metabolizable carcinogens has been demonstrated to be mechanically adequate for treating large numbers of animals. The treatment of hamsters with 0.5% N-nitrosomethylurea (NMU) 2X/week for 15 weeks appears to be adequate for inducing tracheal cancer. A study to determine the effect of 13-cis retinoic acid on NMU-induced tracheal lesions is in progress.

Significance to Biomedical Research and the Program of the Institute: Studies performed under this contract are intended to explore various parameters relating to the induction and prevention of epithelial cancers in animals. Development of new techniques for inducing epithelial cancers in animals may also provide a means for evaluating potential carcinogens to man. The data obtained from the effect of retinoids on various epithelial tumor models will not only evaluate these compounds as chemopreventive agents for different types of epithelial cancers but will also permit structure-function relationships to be determined for the retinoids. These studies will perhaps be of greatest value in establishing a new concept in combating cancer; i.e., chemoprevention.

Proposed Course: Newly synthesized retinoids will be evaluated for chemopreventive activity against respiratory, breast, urinary bladder, and esophageal cancer. In addition, the development and improvement of epithelial tumor models will be continued so that potential anticarcinogenic compounds can be rapidly evaluated.

Date Contract Initiated: June 26, 1972

Current Annual Level: $450,808

ILLINOIS, UNIVERSITY OF (NO1-CP-65843) [Formerly - Case Western Reserve University (NO1-CP-23284)]

Title: Maintenance and Scheduled Sacrifice of Guinea Pigs

Contractor's Project Director: Dr. Samuel S. Epstein

Project Officer (NCI): Dr. Carl E. Smith

Objectives: This project is designed primarily to investigate the carcinogenic potential on F_1 progeny of Hartley guinea pigs of maternal transplacental exposure to NMU and NMUT and to investigate the carcinogenic effects of NMU and NMUT in male Hartley guinea pigs following partial pancreatectomy. Additional studies are performed to investigate the possible in vivo biosynthesis of NMU and its carcinogenic effects following administration of methyl guanidine and sodium nitrite to guinea pigs. Studies were also undertaken to further investigate DNA repair in guinea pig pancreatic slices in vitro and in the pancreas in vivo following exposure to NMUT and HAQO.

Major Findings: No pancreatic tumors have been induced in Contractor's carcinogenesis studies during the past year. However, three uterine tumors (latency period 42-44 weeks) were induced in the female F_1 progeny of guinea pigs which received NMUT during pregnancy and one uterine tumor (latency period 46 weeks) in the control F_1 female group of guinea pigs which received NMUT during pregnancy.

Further studies on DNA repair in guinea pig pancreatic slices in vitro, and in the pancreas in vivo following NMUT or HAQO exposure were based on the analysis of carcinogen-induced DNA damage and subsequent repair on the alkaline sucrose gradient analysis, using preparations of pancreatic nuclei.

In vitro exposure of pancreatic slices to 20 mM NMUT (30 min.) or 10^{-4}M HAQO (15 min.) resulted in a broad slow-sedimenting peak on the gradient of shortened DNA strands. Further incubation of these pancreatic slices for 2-3 hours in a fresh, carcinogen-free, thymidine-containing culture medium demonstrated a shift in the sedimentation of carcinogen-damaged DNA to control sedimentation profiles. These results confirm previous findings on the evidence of DNA damage and repair in pancreatic slices, using hydroxyurea-insensitive thymidine incorporation into DNA as an index of DNA repair synthesis.

Studies on DNA repair in the guinea pig pancreas in vivo were based on the administration to guinea pigs of a single i.p. dose of 30 mg/kg NMUT or 2mg/kg HAQO and alkaline sucrose gradient analysis of isolated pancreatic nuclei for DNA damage and repair, at intervals following carcinogen administration. Immediately (4-16 hours) following carcinogen administration, the pancreatic DNA was severely damaged, as evidenced by the slow-sedimenting DNA peaks on alkaline sucrose gradients. However, at increasing intervals (up to 7-14 days) following administration, there was a gradual shift of pancreatic DNA peaks to control sedimentation profiles, indicating the repair of carcinogen-induced DNA damage in the pancreas.

Significance to Biomedical Research and the Program of the Institute: These studies were designed to develop an animal model to investigate the carcinogenesis of pancreatic tumors and the underlying biochemical mechanism of

carcinogen-nucleic acid interactions. Such studies are considered to be basic to the etiology of pancreatic cancer.

Proposed Course: In the final phases of this contract, the current carcinogenesis studies are being continued for observations on induction of tumors in the pancreas and elsewhere. The animals involved in these carcinogenesis studies will be sacrificed; and, following gross examination, tissues from various organs including the pancreas of the experimental and control groups of the animals will be fixed for slide preparation and detailed histopathological studies of the lesions.

Date Contract Initiated: September 30, 1976

Current Annual Level: $105,209

INDIANA UNIVERSITY (NO1-CP-55654)

Title: Chemical and Structural Requirement for Pancreas Uptake and Excretion: Rats and Guinea Pigs

Contractor's Project Director: Dr. Roger P. Maickel

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To examine the uptake, retention, and excretion of a variety of organic compounds by the pancreas with the intent of characterizing the processes in terms of chemical structures and/or physicochemical properties.

Major Findings: Continuing the studies of plasma and pancreas levels of compounds at 15 and 60 minutes after intravenous administration led to a variety of findings. Initial studies with benzoic acid, benzo(b)thienyl-3-acetic acid, and salicylic acid indicated that while only a modest uptake occurred in the pancreas (tissue:plasma ratios < 0.25), the acidic compounds seemed to be retained to some extent by the pancreas. However, in the case of benzoic acid, when the plasma and pancreas samples were subjected to processing through an extraction procedure specific for the compound administered, no significant retention was observed, suggesting that the radioactivity retained by the pancreas represented metabolite(s). A variety of basic compounds, including decamethonium, isoniazid, D- and L-methadone, and propranolol all showed significant pancreatic uptake; and, in the cases of decamethonium, isoniazid, and D-methadone a significant tendency for pancreatic retention. Application of specific assay procedures confirmed these findings for L-methadone and propranolol; the pure compounds show a greater tendency to remain in the pancreas than do the metabolites. Studies of disposition of basic compounds such as amphetamine, decamethonium, methadone, and propranolol indicate a considerable degree of localization in other tissues as well. A method has been developed for collecting pancreatic secretions in the rat. Preliminary studies using this procedure confirm that compounds such as decamethonium and methadone are secreted by the rat pancreas.

Significance to Biomedical Research and the Program of the Institute: The results obtained to date indicate that the rat test system is a useful technique for measuring pancreatic uptake and retention of organic compounds. The characteristics of a given compound that determine this pancreatic uptake and retention should be applicable to permit generalization to other compounds and lead to the rational design of compounds to experimentally induce pancreatic neoplasms, to yield valuable information regarding their growth, and to act specifically as organ-selective chemopreventive or therapeutic agents.

Proposed Course: Studies are now beginning to examine the accumulation of compounds by the pancreas (in contrast to other tissues) on repetitive administration of selected model compounds for 5-15 days. Methodology will be used to permit quantitative determination of authentic compounds as opposed to total radioactivity; the role of metabolites will be determined. In addition, the pancreatic secretion technique will be applied to the study of selected compounds to test whether retention accumulation and selective secretion occur with chronic dosage.

Date Contract Initiated: June 30, 1975

Current Annual Level: $107,680

KAISER FOUNDATION RESEARCH INSTITUTE (NO1-CP-33215)

Title: Epidemiologic Study of Colorectal Cancer in Blacks

Contractor's Project Directors: Dr. Gary D. Friedman
Dr. Loring G. Dales

Project Officer (NCI): Dr. Carl E. Smith

Objectives: This is a case-control study of diet and colorectal cancer among San Francisco Bay Area Blacks, exploring potential associations between colorectal cancer and intake of meats (particularly beef), saturated fats, foods treated with nitrite preservatives, and staple foods low in unrefined carbohydrate or roughage. Several other traits are also studied for potential association with bowel cancer.

Major Findings: Data collection has been completed. Colorectal cancer cases (105) and approximately three times that number of controls were interviewed. Data analysis is nearing completion. Frequency of beef consumption shows no real relationship to colorectal cancer. Frequency of consumption of foods high in saturated fats, of meat in general, and of foods treated with nitrite preservatives show small, statistically non-significant, positive associations with cancer of the colon and rectosigmoid junction. Frequency of consumption of foods high in unrefined carbohydrate or roughage shows a small, statistically non-significant negative association with colon cancer. Two non-dietary traits, history of diarrhea or loose/watery stools and, among women, nulliparity show statistically significant

($p<.01$), positive associations with colorectal cancer. Further analysis of all of these associations is in progress.

Significance to Biomedical Research and the Program of the Institute: Hypotheses relating consumption of saturated fat, meat, and indigestible fiber to colorectal cancer causation have been advanced, and experimental and "metabolic epidemiology" data bearing on these hypotheses are accumulating. Epidemiologic data on associations between these dietary traits and colorectal occurrence are rather sparse and inconsistent. The hypotheses thus need further corroborative or refutative evidence.

Proposed Course: Completion of data analysis in March, 1977

Date Contract Initiated: December 1, 1972

Current Annual Level: 0

LITTON BIONETICS, INC. (NO1-CP-65847)

Title: Resource for Microscopic and Autoradiographic Technology

Contractor's Project Director: Dr. Elizabeth W. Kingsbury

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: Light and electron microscopy and high resolution light autoradiography (ARG) will provide morphological data on the pathogenesis of tumors of various target organs, such as, the colon, pancreas, lung, prostate and breast, and data on the localization of labeled compounds, including carcinogens, in cellular organelles.

Major Findings: This contract has only recently been awarded; however, two tasks have already been initiated. These two projects are 1) "Light Microscopic, Electron Microscopic, and Autoradiographic Investigations in Support of Pathogenesis Studies of Pancreatic Adenocarcinoma" and 2) "Identification of Cultured Cells Isolated from Human and Animal Tissues." It is too early for results to be available.

Significance to Biomedical Research and the Program of the Institute: As the incidence of cancer is on the rise in our society, it has become necessary to understand the mechanism of carcinogenesis and the effects of increasing levels of carcinogens in our environment. Developing model systems and determining the effects of carcinogens on these systems are important aspects of understanding carcinogenic processes. The studies to be performed are significant in that they are geared towards characterizing these processes.

Proposed Course: The two projects indicated above will be completed and further support efforts initiated as required.

Date Contract Initiated: September 21, 1976

Current Annual Level: $28,209

LITTON BIONETICS, INC. (NO1-CP-43274)

Title: Studies of Carcinogenesis in Human Bronchial Tissues

Contractor's Project Director: Dr. Marion Valerio

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: To use the athymic nude mouse as an animal model for long-term survival of xenografts in order to study the reaction of human tissues (bronchus, pancreatic duct, colon) exposed to various carcinogens and/or anticarcinogens prior to or at the time of implantation.

Major Findings: The modified barrier facility housing the nude mouse breeding colony and experimental mice has proven adequate for long-term survival. Several nude mice retired from the breeding colony have survived for over 2 years. Minor bacterial contamination did occur within the colony, but, according to the viral screening assay system used, the colony has remained free of PVM, Reo-3, GDVII, Polyoma, Sendai, MVM, MAd, MHV, and LCM.

The breeding colony has produced sufficient athymic nude mice for the experimental purposes of this contract until this time. The athymic nude mouse kept under these conditions will support human bronchial tissue xenografts for over 1 year. During the past year bronchi from 26 individuals were implanted subcutaneously into 225 mice. Human colon was implanted subcutaneously or intraperitoneally into 118 mice. Survival and growth of colonic epithelium has not been successful. Human and bovine pancreatic duct segments have been xenografted and have survived.

Xenografts have been exposed in situ to 7,12 dimethylbenz(a)anthracene (DMBA) and/or amosite asbestos which caused basal cell hyperplasia and atypical squamous cell metaplasia in the bronchial epithelium. Insertion of the beeswax pellets containing high levels of DMBA (100, 330, 1000 μg) at the time of xenografting has also caused sarcomas of mouse origin apparently from leakage of the carcinogen; insertion of the DMBA pellet in the bronchial lumen one month after xenografting may have eliminated this problem. Another approach currently being investigated is to use chemicals, such as aflatoxin B_1, which are metabolically activated by cultured human bronchi but are not carcinogenic in the adult mouse. Polonium 210 adsorbed on particulates of ferric oxide is also being used to expose bronchial xenografts. Since alpha irradiation from PO^{210} penetrates the tissue only a few microns, it is anticipated that only the human bronchial epithelium will be irradiated. In addition, experiments are ongoing in which human tissues are exposed to chemical carcinogens such as MNNG in vitro, transplanted into athymic nude mice, and then pellets with/without carcinogens are inserted one month after the xenograft.

A small outbreak of a condition not previously diagnosed occurred in the mouse colony. Eleven mice died of hemothorax within a ten-day period. All were males between 202 to 547 days of age. The condition has been postulated to be nutritional and due to a vitamin K deficiency. The diet was changed, but the condition disappeared spontaneously at the same time.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal host for human bronchial tissue xenografts which have been treated with carcinogens and/or anticarcinogens in vitro and/or in vivo will provide a model more predictive of the effects of carcinogens and anticarcinogens on human tissues than extrapolation from an animal tumor or tissue culture model system.

Proposed Course: Continue to implant xenografts of human bronchial tissue in the athymic nude mouse. The effects of various chemical carcinogens and alpha radiation will be tested using this system. Dose response studies will continue with the various tissues. Continue work begun with human and bovine pancreatic ducts. Establish methods for routinely and quickly determining tissue origin (human or mouse) of tumors produced. Expand the nude mouse breeding colony to produce more weanlings for experimental purposes.

Date Contract Initiated: February 1, 1974

Current Annual Level: $151,293

MARYLAND, UNIVERSITY OF (NO1-CP-43237)

Title: Studies on Carcinogenesis in Human Tissues: Bronchial Epithelium, Pancreas, Breast, and Colon

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) Acquire normal, preneoplastic, and malignant human tissue for in vitro studies; (2) study morphological and cytochemical parameters of human bronchial epithelium, pancreatic duct, breast, and colon tissue before, during, and after growth in explant culture; and (3) explore tissue responses to application of selected carcinogens.

Major Findings: Bronchial Epithelium: (1) Morphological (including transmission and scanning electron microscopy, (TEM, SEM)) and histochemical studies of normal, abnormal, premalig nt, and malignant epithelium are continuing; (2) Eighty lung tumors have been examined, and a new histogenetic classification has been developed based on more severe criteria than are required for present classification schemata. Correlative cytological studies are being made. Studies are in progress to examine the effects of continous exposure of the epithelium to benzo(a)pyrene over a two-month period in explant culture.

Inducibility of aryl hydrocarbon hydroxylase (AHH) in bronchial epithelium of human, rhesus monkey, cow, and dog in explant culture has been established. The activity of the induced enzyme has been shown to be proportional to the inducer concentration (benz[a]anthracene and 3-methylcholanthrene). AAH inducibility has also been established in human peripheral blood monocytes obtained from healthy human volunteers. This provides a means for correlation between AHH inducibility in the readily obtainable peripheral cells and in the target tissue.

Clinical histories have been extracted from hospital medical records of 50 patients with and without lung cancer. These patients, from whom bronchial tissue was resected, have also answered a questionnaire. Work is in progress correlating the results of carcinogen binding to bronchus in vitro with tumor type and with epidemiological data derived from questioning the patients.

Pancreatic Epithelium: 1) Human pancreatic duct has been maintained for up to 60 days in explant culture with preservation of viability as measured morphologically and cytochemically. Bovine pancreatic duct, cultured for 90 days, was utilized initially to develop methodology. 2) Studies are in progress using human pancreatic ductal explants exposed to MNNG. The tissues are being examined using morphological and selected histochemical methods. The changes occurring in explant culture are continuously monitored by cytological methods; i.e., Papanicolau staining.

Mammary Epithelium: 1) Morphological studies including light microscopy, TEM and SEM of normal, abnormal, premalignant, and malignant epithelium are continuing (59 cases). Normal tissue is obtained from immediate autopsy and reductive mammoplasty patients and premalignant and malignant tissue from patients undergoing mastectomies. 2) Explant cultures are made at the University of Maryland and monolayer cultures are being prepared in collaboration with Dr. Janss at the Frederick Cancer Research Center. Normal human breast epithelium has been maintained in explant culture for 133 days, with preservation of viability. It appears that the age of the patient contributes to the length of viability in explant culture.

Colonic Epithelium: 1) Human colon samples from normal, preneoplastic, and malignant tissue, acquired at surgery and immediate autopsy, have been prepared for microscopy (84 cases). All cases have been studied by light microscopy. Most malignant, some preneoplastic, and many "normal" colon samples have been examined by TEM. In addition, a number of cases have also been examined by SEM, looking at both luminal surfaces and the surfaces of cross-sectional slices. The morphological and cytochemical characteristics of these various tissues are being carefully evaluated; 2) Human colonic epithelium, acquired at surgery and immediate autopsy, has been maintained in explant culture for a long as eight days. Viability was monitored by ultrastructural morphology. The best culturing conditions included using CMRL 1066 media with high glucose concentration at 37°C and a gas phase of 95% O_2-5% CO_2. In an effort to prolong the time in culture, other experimental conditions are being tested.

Collaborative Studies: In addition to the above studies performed at the University of Maryland, human tissues are supplied to Dr. Harris at the National Cancer Institute for carcinogenesis experiments including the metabolism of various carcinogens by human tissues in vitro.

Significane to Biomedical Research and the Program of the Institute: The importance of this project lies in the fact that the nature of premalignant changes in human bronchus, pancreas, colon, and breast are not well understood,and little is known concerning carcinogen binding and the response to carcinogens of human tissues. A number of observations have been made which ultimately are expected to offer to the study of carcinogenesis new tools and resources in its search for identification of the key events leading to induction of cancer. These studies, it is hoped, will be of significance in understanding the process of carcinogenesis and will establish models for future studies in these and other organ systems.

Proposed Course: (a) To further determine the nature of the cellular response to carcinogens in the human bronchus; (b) to further establish a classification of lung tumors and correlate with clinical findings and epidemiological factors; (c) to examine the metabolic and morphologic response of human bronchus to carcinogens in vitro and in vivo and to correlate this response with smoking and occupational histories, as well as with carcinogen metabolism by other cells such as peripheral blood monocytes; and (d) to perform similar studies on breast, pancreas duct, and colon mucosa.

Date Contract Initiated: October 1, 1973

Current Annual Level: $287,100

MAYO FOUNDATION (NO1-CP-55660)

Title: Reflux as a Mechanism for Induction of Adenocarcinoma of the Pancreas

Contractor's Project Director: Dr. Eugene P. DiMagno

Project Officer (NCI): Dr. Carl E. Smith

Objectives: (1) Examine at autopsy the anatomical relationships of the human pancreatic duct, bile duct, and duodenum and relate this arrangement to duct cell histology; (2) determine if there are relationships between epidemiological variables such as sex, smoking habits, alcohol intake, lipoprotein patterns, and duct cell histology; (3) develop an animal model for induction of pancreatic duct reflux; and (4) examine human pancreatic secretion for potential carcinogens.

Major Findings: The anatomical relationships between the pancreatic duct, bile duct, and duodenum are being done in human autopsy specimens in which these organs are dissected en bloc. A procedure has been developed to

radiographically record anatomical relationships and, at the same time, examine pancreatic ductal histology. The major and minor papillae and/or entry for common bile duct are identified and injected with a dilute radio-opaque barium-formaldehyde mixture. After injection, a stereoscopic radiograph is taken. Then a probe is placed in the main pancreatic duct, the pancreas filleted, and the filleted pancreas photographed. Longitudinal microscopic sections are made of the entire pancreatic duct. Contractor also obtains information regarding smoking and alcohol habits as well as serum triglycerides, cholesterol, routine laboratory data, occupation, sex, age, cause of death, and all other diagnoses from the clinic record.

To date, 262 autopsies have been performed and 115 analyzed. Although data is incomplete, there appears to be a trend toward an association between non-common channel ductal arrangements and abnormal pancreatic duct histology. Of the 115 autopsies, in 97 the entry of ducts into the duodenum could be determined: common channel was present in 39, an interposed septum between the common bile duct and pancreatic duct in 37, and separate entry of the common bile duct and pancreatic duct in 21. A higher percentage of abnormal ductal histology occurred (46 and 38%) in pancreatic ducts that had interposed septum or entered separately compared to common channel (28%).

A canine model (comprised of a chronic gastric fistula and a permanent pancreatic duct - cutaneous catheter) has been developed which allows simultaneous quantification of pancreatic enzyme outputs, duodenal volume flow, and pancreatic and duodenal pressures under physiologic circumstances. Twelve studies demonstrated that mean fasting pancreatic pressure was 5 to 10 cm. H_2O higher than mean fasting duodenal pressure. Further, 8 minutes after ingestion of a meal, both the pancreatic and the duodenal pressures increased. Surprisingly, the mean duodenal pressure was higher than the mean pancreatic pressure 20 minutes after feeding. This relationship lasted for 30 minutes. Lastly, elevation of postprandial pancreatic pressure occurred concomitantly with increased enzyme output, and duodenal pressure increased with increased duodenal volume flow. In recent experiments, reflux of duodenal contents into the pancreatic duct has been observed. A small amount (< 1%) of a radioactive nonabsorbable marker perfused into the duodenum is recoverable from the pancreatic duct at postprandial periods when duodenal pressure exceeds pancreatic duct pressure.

Significance to Biomedical Research and the Program of the Institute: Human pancreatic cancer predominantly arises from the ducts of the head of the pancreas, and reflux of biliary or duodenal contents into the pancreatic duct has been suggested as a possible mechanism of pancreatic carcinogenesis. This preliminary autopsy data suggests there may be a relationship between the anatomical arrangement of the entry of the bile and pancreatic ducts into the duodenum (separate entry of ducts or an interposed septum) and induction of abnormal histologic changes of pancreatic ductal epithelium. The anatomic relations which appear to be associated with abnormal histology are those in which the pancreatic duct enters directly or approximates direct duct entry into the duodenum (similar to Contractor's canine model).

Secondly, an animal model has been developed in which large duodenal volume flows associated with duodenal pressures greater than pancreatic duct pressure may induce reflux of duodenal contents into the pancreatic duct. Recent experiments suggest that reflux, although small, may occur.

Proposed Course: The autopsy studies outlined above will continue in an attempt to determine whether anatomical relationships of the pancreatic duct and duodenum are associated with abnormal pancreatic duct histology and whether possible epidemiological factors can be related to pancreatic cancer (preliminary evidence suggests that smoking is). Definitive studies in the canine model will continue employing hormones, drugs, and measurements of sphincter mechanisms to better delineate the existence and physiology of reflux into the pancreatic duct. In collaboration with Dr. Thomas Spelsberg of this Institution, measurements will be made of potential carcinogens from cigarette smoke in pancreatic secretions of the human and canine animal model.

Date Contract Initiated: June 30, 1975

Current Annual Level: $117,800

MELOY LABORATORIES, INC. (NO1-CP-55613)

Title: Transplacental Carcinogenesis in the Old World Monkey, Erythrocebus Patas

Contractor's Project Director: Dr. D. Lewis Sly

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: This project is designed to provide a nonhuman primate model which will demonstrate transplacental chemical carcinogenesis.

Major Findings: Exposure to 1-ethyl-1-nitrosourea (ENU) in three age groups of patas monkeys has resulted in tumor development in all three age groups. Seven of thirteen juvenile monkeys have yielded nine cancers of differing histologic types and tissues of origin. One breeding female was found to have multiple epithelial tumor nodules in the lung subsequent to her third pregnancy during which ENU was administered. Three transplacentally exposed baby monkeys have developed tumors. One baby died at seven months of age with a large leiomyosarcoma of the lung, another at two months with a hepatocellular adenoma of the liver and a hemangiosarcoma of the brain. The third animal was born with a malignant hemangioendothelioma of the face. The appearance of tumors at such a short time after birth is quite different from rodent models in which transplancentally exposed animals develop tumors in young adult life. The tumor specturm observed so far resembles that associated with pediatric neoplasms in man in contrast with rodent models which yield chiefly epithelial and neuroectodermal tumors of adult morphology.

Significance to Biomedical Research and the Program of the Institute: With recognition of the association between exposure to diethylstilbestrol in utero and development of vaginal adenocarcinoma during the second decade of life, the possible significance to human health of transplacental chemical carcinogens has become increasingly a matter of concern. Experimental studies on transplacental chemical carcinogenesis have been limited to rodent species, which may differ significantly from man in ways likely to be of importance for understanding the different consequences of exposure to chemical carcinogens during fetal versus adult life. Among these differences are the more rapid rates of fetal neonatal development and maturation in rodents.

Proposed Course: Particular emphasis will be placed on defining the periods of maximal sensitivity to ENU and diethylnitrosamine (DEN) in different organ systems of the fetus. Comparative studies on tissue damage and subsequent DNA repair as they relate to carcinogenesis in the patas monkey and in rats and mice will be initiated.

Date Contract Initiated: October 23, 1974

Current Annual Level: $211,666

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-43366)

Title: Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant, and Malignant Colonic Tissues in Familial Polyposis

Contractor's Project Director: Dr. Martin Lipkin

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To develop indices that define the phenotypic stages of neoplasia in cells of a high risk human population. Cells derived from individuals with the dominant inherited disease familial polyposis are studied for changes in growth characteristics and related biochemical parameters, and affected individuals are classified on the basis of the findings.

Major Findings: Studies on phenotypic abnormalities in skin fibroblasts (SF) derived from individuals with familial polyposis were continued. In familial polyposis, increased susceptibility of SF to transformation by Kirsten murine sarcoma virus was observed. Transformed human SF were positive to murine leukemia group-specific (GS) antigen in cytoplasm of infected cells and contained RNA-dependent DNA polymerase. Studies of virus adsorption, production of infectious centers on indicator rat NRK cells, and XC syncytial assay on human SF post infection were similar in polyposis and control cells.

In studies of chromosomal proteins of SF investigated by electrophoresis in SDS gels, chromosomal proteins from desmoid tumor differed from SF derived from flat skin; electrophoretic profiles of chromosomal proteins of SF strains from flat skin had similar characteristics although there were

variations between SF. Initial measurements of distribution of actin cables in SF from polyposis and control groups suggested that SF from normal individuals contain more actin cables than polyposis SF.

Fragments of human colonic adenomas were implanted under the renal capsule of athymic nude mice and survived for at least one month. Specimens retained original structure with identification of adenomatous tissue. The familial polyposis resource was used to study fecal neutral steroids and bile acids in collaboration with American Health Foundation (NO1-CP-33208). Although total neutral sterol concentrations were not different between groups, individuals with familial polyposis excreted a high amount of cholesterol and low levels of coprostanol and coprostanone compared to controls.

Significance to Biomedical Research and the Program of the Institute: These findings identify phenotypic expressions developing in human cells as they undergo neoplastic transformation. They provide indices to detect individuals with expressed or latent inherited polyposis, a disease leading to colon cancer. The results enable well-defined human cells to be used in studies to identify potential carcinogens relevant to colon cancer. They provide a means to study the interaction of inherited and environmental factors in carcinogenesis. The results also provide a well-defined human population group to be used in experiments designed to prevent further stages of neoplastic transformation in colonic cells of man.

Proposed Course: Studies will be continued to develop markers or indices defining the stages of preneoplasia in cells of this human population group at high risk of colon cancer. Individuals and population groups will be classified on the basis of cell phenotype and related abnormalities to identify heightened degrees of susceptibility of individuals at increased risk of colon cancer, the stages of development of their disease, and the potential contribution of elements that can accelerate or modify the progression of disease in man. Studies will be initiated to investigate the interaction of chemical carcinogens with nuclear RNA and to study the effects of this interaction on RNA processing.

Date Contract Initiated: October 15, 1971

Current Annual Level: $203,025

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-43232)

Title: Identification of Site of Origin of Human Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Patrick J. Fitzgerald

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To identify the cell origin of adenocarcinoma of the pancreas using light microscopic, histochemical, and electronmicroscopic techniques.

Major Findings: Contractor has studied 42 major recently-resected specimens of patients with adenocarcinomas in the region of the head of the pancreas and the biliary-ampullary-duodenal area. By doing a careful gross examination and by taking multiple sections of the different anatomic compartments converging on this region, contractor was able to classify these tumors according to the site of origin, as follows: pancreatic duct carcinoma 24 (57%), ampullary carcinoma 7 (17%), duodenal carcinoma 3 (7%), bile duct carcinoma 3 (7%), and carcinoma of undetermined origin 5 (12%).

Tissue for electronmicroscopic studies was taken in 36 of the above described cases and in 30 (83%) mucigen could be demonstrated.

It was concluded that a careful gross and microscopic study of a resected tumor in the pancreatoduodenal region will permit a correct classification of tumors of this area in about 80% of the cases.

Electron microscopic examination of over 100 cases of cancer of the head of the pancreas region, including pancreatic, ampullary, duodenal, and bile duct tumors, demonstrated that most of these cancers were adenocarcinomas of ductal origin because of the presence of mucigen granules and the absence of zymogen or islet cell granules. Over 80% of pancreatic duct cancers were shown to contain mucigen granules. Electron microscopic study of carcinomas in this area has been useful in distinguishing endocrine from nonendocrine cancers and pancreatic from ampullary or duodenal carcinomas. There is, however, a small group of far advanced cases in which classification as to exact site of origin is not possible.

A clinical and pathologic study of 31 cases of periampullary carcinoma diagnosed at Memorial Hospital was performed. A new classification based on clinical, gross, and histologic features was devised in order to separate duodenal and periampullary tumors from pancreatic duct cancers. The most common type of periampullary cancer was the intra-ampullary variety [17 cases, (55%)] followed by the exophytic type [9 cases, (29%)] and a mixed group, ampullary-exophytic [5 cases, (16%)].

It appears that intra-ampullary tumors originate in ampullary tissues proper and that exophytic lesions probably have a duodenal origin. More than one site of origin could be present in the mixed ampullary-exophytic group.

In carcinomas of the periampullary region, atypical hyperplasia and carcinoma in situ of ampullary or duodenal tissue was found in 50% of the cases. In 50% of the cases, those changes were multifocal in more than one type of tissue (duodenal, ampulla, pancreatic duct, or bile duct).

The patterns of spread of duct cell adenocarcinoma was studied in a group of 380 patients seen at Memorial Hospital during the period 1949-1972.

Peripancreatic lymph nodes, adjacent organs, and liver were common sites of tumor extension at surgery and at autopsy. Carcinomas of the head of the pancreas tended to spread more locally and cancers of body and tail more diffusely.

An anatomical study of normal distribution of lymph nodes and their metastatic involvement was performed in 33 patients with carcinomas arising in the pancreas or periampullary region. The classification employed was the Superior Group: superior, head (SH), superior, body (SB); Posterior Group; and Inferior Group: inferior, head (IH), inferior, body (IB). Pancreas duct cancer metastasis was most common in the Superior Group [17 patients, (52%), (10 SH, 7 SB, and 4 with both divisions)] and the next most frequent were those in the Posterior Group [10 patients, (30%)]. There were 7 patients (21%) with positive nodes in the Inferior Group (6 IH only and 1 IB only). In 10 patients (30%), metastasis was present in only one group of lymph nodes (Superior Group 7, Posterior Group 2, and Inferior Group 1). Metastases were present in 2 lymph node groups in 4 patients (12%) and in more than 2 groups in 5 patients (15%). No metastasis was noted in 3 patients (9%).

Ampullary and duodenal cancers metastasized less frequently and less widely than pancreas duct cancers, usually to the APD, PPD (anterior and posterior pancreaticoduodenal, respectively), SH, and IH groups of nodes, and they had fewer positive nodes than the pancreas cancers.

Significance to Biomedical Research and the Program of the Institute: The finding that about 90% of pancreas cancers are of ductal epithelium should focus the attention of investigators on the duct epithelium of the pancreas. There is some information available at the present time on the ultrastructure, histochemistry, and biochemistry of the normal pancreatic duct epithelium but relatively little compared to acinar and islet cells.

The presence of atypias and carcinoma in situ in the ducts of about 20% of cases of pancreas adenocarcinoma, and none in a non-cancer of a control group, would indicate that these lesions are possibly preneoplastic changes. This would also suggest that these may be a latent period between the recognizable onset of the cancer and the appearance of invasive lesions, as with many other cancers. If this is so, an effort should be made to define populations at risk so that an earlier diagnosis may be made in them through new techniques, such as, pancreatic duct cannulation, cytology of duodenal aspirates, computerized transaxial tomography (CCT) (the body scanner), sonography, and others.

The finding of carcinoma in situ and atypical hyperplasia in 50% of patients with periampullary carcinoma is of interest, particularly in view of the 20% incidence in pancreas duct regions. It is possible that the difference in the frequency of the changes may be because there is better sampling of the smaller ampullary epithelium. Possibly the larger pancreas cancers at the time of diagnosis may have overgrown some in situ lesions.

The presence of multifocal in situ carcinoma lesions in different structures in the pancreatico-biliary-ampullary-duodenal tree is similar to the multifocal nature of cancers of the oropharynx, respiratory tract, and other organs.

The precise morphologic classification of tumors of the pancreatico-biliary-ampullary-duodenal region may be important because tumors arising in different anatomical sites have a different biologic behavior. It is also possible that these tumors are the result of different carcinogenic substances.

The high degree of nerve sheath involvement may suggest that widespread metastases may originate through this route of spread.

Proposed Course: Objectives in this contract have been reached in a most successful manner. The contract is scheduled for termination on May 31, 1977.

Date Contract Initiated: June 1, 1974

Current Annual Level: 0

MICHICAN, UNIVERSITY OF (NO1-CP-55720)

Title: Organ Culture of the Prostate: A Biological Model

Contractor's Project Director: Dr. Raymond H. Kahn

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To determine the requirements, both physical and chemical, for maintaining normal dog and human prostate glands outside the body as organized tissue (organ culture) and to establish the structural criteria for normal prostatic tissue.

Major Findings: Canine prostate can be cultivated in a well-maintained state for 10 days in vitro if the donor dog has been pretreated with methylprednisolone before prostatectomy and if the explants are exposed to vitamin A in culture. Testosterone is ineffective in epithelial maintenance in vitro, as is 5α-dihydrotestosterone. Explants are not maintained, however, if cultivated in media containing serum. Prolactin, polyamines, linoleic acid, and vitamin E were all found to have no histological effect on epithelial maintenance.

Significance to Biomedical Research and the Program of the Institute: Although prostatic cancer is one of the leading causes of death in elderly men, there is no available model systems for studying the normal prostate nor the natural history of change with age. While the dog seems to be the most closely related model, investigations using the intact animal are both expensive and time-consuming. Moreover, in the intact animal, it

is also difficult to distinguish changes in the prostate due to experimental regimen from those due to organ interactions, serum hormone levels, and other factors. The development of a biological model maintained in organ culture would permit the replication of the body's response under carefully controlled conditions, divorced from organ interactions. Such a model would lend itself to an assessment of the cause of prostate cancerous changes (carcinogenesis), development of possible preventive measures, and evaluation of treatment protocols. Once a model system has been established, early diagnosis of lesions also would be conceivable.

Proposed Course: During the remainder of the contract period, experiments will be done to investigate the effect of testosterone metabolites, especially 5α-androstan-3α,17α-diol which has been implicated as the "active" canine prostatic androgen in both in vivo and in vitro studies. Exhaustive analysis of the experiments referred to above will continue to detect any possible treatment interactions.

Date Contract Initiated: June 25, 1975

Current Annual Level: $19,500

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-02199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contractor's Project Director: Dr. Martin L. Wenk

Project Director (NCI): Dr. Jerry M. Rice

Objectives: This contract provides laboratory support services and collaborative research technology for the Carcinogenesis Program of NCI. Experimental protocols developed in collaboration with NCI investigators are carried out through the use of contract facilities for the following functions: (1) holding, treatment, and observations (including gross pathological evaluations) of small rodent species; (2) collection, preservation, and histological processing of tissues as specified by individual protocols; (3) application of biochemical techniques for the determination of events associated with chemical carcinogenesis in various model systems; (4) maintenance and treatment of cultured cells or tissues; (5) production of 1μ and ultra-thin section for high resolution light microscopy; and (6) processing of 5μ and 1μ tissue sections for autoradiography.

Major Findings: During the past year, collaborative studies have involved 42 individual experimental protocols designed jointly by the NCI and Microbiological Associates, Inc. investigators. Contract efforts were concentrated within the following areas: (1) Development of in vivo and in vitro model systems for the study of chemical carcinogenesis including: a) in vitro assays for chemical transformation; b) in vivo transplantation

assays for malignant transformation; c) development of an animal model for induction of pancreatic carcinoma; d) development of in vitro assays for evaluation of anti-tumor effects of synthetic retinoids; e) documentation of increased genetic sensitivity to skin carcinogenesis in mice; f) development of transplacental carcinogenesis models for neurological tumors; and g) development of animal models for gastrointestinal tumors. (2) Utilization of existing models for studies involving the documentation of subcellular events associated with chemical carcinogenesis, including expression of endogenous viruses. (3) Utilization of model systems for histogenesis studies of chemically induced tumors including: a) esophageal tumors and b) pancreatic tumors. 4) Utilization of existing models for evaluations of factors modifying chemical carcinogenesis including: a) anti-tumor potential of synthetic vitamin A analogues in bladder, colon, and pancreatic tumor formation and b) evaluation of possible cocarcinogenic activity of asbestos in various organ systems. 5) Utilization of contract capabilities for service in support of NCI research programs including animal holding, supply of treated or untreated experimental animals as dictated by approved protocols. All resulting publications are coauthored by Contractor and NCI staff.

Significance to Biomedical Research and the Program of the Institute: This contract offers the ability to conduct long-term experiments involving large numbers of experimental animals, the ability to conduct rapid tests of new approaches in experimental pathology related to chemical carcinogenesis, and the ability to follow up rapidly on studies that demonstrate relevance to the overall objectives of the Carcinogenesis Program, NCI.

Proposed Course: This contract will continue to offer a diversified facility for the support of long-term studies in chemical carcinogenesis orginating from the NCI Carcinogenesis Program.

Date Contract Initiated: June 4, 1970

Current Annual Level: $750,000

MINNESOTA, UNIVERSITY OF (NO1-CP-55702)

Title: Rhythmometry on Japanese and North American Female Volunteers of Different Age Groups

Contractor's Project Director: Dr. Franz Halberg

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: To determine the sampling requirements in approaching the problem of whether or not women with different risk for breast cancer exhibit differences in the concentration of 13 different blood hormones—when variability is systematically assessed by the study of a spectrum of rhythms—including, for all variables, some changes with frequencies ranging from one cycle in a few hours (ultradian rhythms) to one cycle in a year (circannual rhythms). To assist in evaluating hormonal variations in blood, rhythms in 5 hormonal variables in urine, and those of other variables including motor body activity, body core temperature, and blood pressure

also are to be monitored in postpubertal, young adult and postmenopausal women in different geographic locations, including Japan and the U.S.A., with focus upon ultradian, about 24-hour (circadian), about weekly (circaseptan), about monthly (circatrigintan) and circannual rhythmic components.

Major Findings: Some criteria for subject selection had to be compromised, because of the paucity of volunteers satisfying all risk and general health criteria simultaneously; family history of breast cancer and age at first pregnancy remain the primary risk factors taken into consideration. Consideration of addition of subjects beyond the originally stated age group, as proposed earlier, seems extremely desirable if not essential. In any event, recruitment in the originally visualized categories will continue beyond the borders of Minnesota. Subjects from Chicago, for instance, have come for screening as potential volunteers.

The major portion of sampling for the first quarter of the study has been completed; the loss of 6 subjects (for various medical and personal reasons) will necessitate the extension of sampling (for at least the 6 new replacements, who will have their first sampling in the second or third quarter). In Japan, the first quarter of sampling is also completed; shipment of specimens has been without incident thus far.

The radioimmunoassays for a number of hormones are in progress; those on 4 Minnesotan subjects completed, thus far, have been extensively analyzed; results demonstrate, first and foremost, that on an individual basis, a statistically significant circadian rhythm could be quantified for cortisol, T4 and prolactin.

Significance to Biomedical Research and the Program of the Institute: The difference in incidence of breast cancer between the United States and Japan is well-documented. Numerous explanations have been offered for this observation all of which may play a discrete but significant part in the carcinogenic process. One pathway for genetic, environmental, or sociological effects may involve the mediation of blood hormone concentrations in healthy women, as these are influenced by cyclic physiological processes.

The results of this project are expected to quantitatively define the extent of the rhythmicities affecting individual hormone concentrations in high and low risk populations. On this basis, a reasonable protocol to assess the hormonal milieu of an individual without continuous or constant daily surveillance may be planned.

Proposed Course: The radioimmunoassays of 13 hormones, followed by computer analysis to detect the characteristics of any statistically significant rhythms, will continue on the samples collected during the first quarter. Comparison of these characteristics will be made between risk groups of different ages.

Date Contract Initiated: June 30, 1975

Current Annual Level: $148,033

MINNESOTA, UNIVERSITY OF (NO1-CP-33364)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: It has been demonstrated that several types of antioxidants will inhibit pulmonary neoplasia resulting from administration of polycyclic hydrocarbons. Specific objectives relevant to these observations are: (1) to elucidate the mechanisms of this inhibition, (2) to determine the conditions under which inhibition occurs, (3) to ascertain optimal methods for utilizing antioxidants and related compounds to inhibit pulmonary neoplasia, and (4) to define relationships between chemical structure and the capacity of a compound to inhibit pulmonary neoplasia. In a second part of the proposed work, objectives are to determine the relationship between the activity and compostion of the aryl hydrocarbon hydroxylase system and the binding of metabolites of polycyclic hydrocarbons to macromolecules of pulmonary structures and, in addition, to attempt to relate the amount of binding to the development of pulmonary neoplasia.

Major Findings: Investigations of the microsomal metabolism of BP have been carried out in animals which have received BHA under conditions which inhibit BP-induced pulmonary neoplasia. Two sets of studies of this type have been carried out. In one the animals have been given BHA in the diet, and in the second, they have been given BHA by oral intubation. In both instances, there are marked changes in the metabolism of BP in microsomes of the animals which have received BHA. The metabolites of tritium-labeled benzo(a)pyrene incubated with microsomes from animals which had received BHA and comparable controls were analyzed by high pressure liquid chromatography. The administration of BHA resulted in profound alterations in the metabolite pattern observed. These alterations could be divided into two categories, both of which would result in a decrease in the carcinogenic effect of BP. The first category was an increase in 3-hydroxybenzo(a)pyrene, a metabolite of detoxification. The second category was a decrease in epoxidation which is a process necessary for the metabolism of benzo(a)pyrene to its ultimate carcinogenic form. These data indicate that BHA is exerting its inhibitory function by altering microsomal metabolism of the carcinogen. In the studies in which BHA has been given by oral intubation, it was found that the changes in metabolite pattern occurred as early as two hours after administration of the antioxidant. This short time interval indicates that the BHA is having a direct effect on the microsomal enzymes metabolizing BP.

Significance to Biomedical Research and the Program of the Institute: Man now consumes a substantial amount of antioxidants. In animal models these compounds have been shown to have the capacity to inhibit chemical carcinogenesis. It is of importance to know the mechanism of this inhibition and to evaluate the present and potential role of antioxidants as inhibitors of the effect of chemical carcinogens in the environment on pulmonary

neoplasia. Such information could be of importance in minimizing the impact of environmental carcinogens on pulmonary neoplasia in man.

Studies on the relationship between the activity and characteristics of the aryl hydrocarbon hydroxylase system and the development of pulmonary neoplasia comprise the second portion of the present project. The AHH system is subject to alteration in activity and characteristics by pure compounds as well as dietary constituents. It is of importance to determine how these alterations may affect the susceptibility of the host to neoplastic response from chemical carcinogens.

Proposed Course: The proposed course includes studies of the mechanism of antioxidant inhibition of polycyclic hydrocarbon carcinogen-induced pulmonary neoplasia. Accompanying animal studies will determine conditions under which neoplasia is inhibited and the relationships between chemical structure and capacity of compounds to exert inhibitory effects. Investigations are also in progress to determine the relationship between activity and characteristics of the AHH system and the extent of macromolecular binding of carcinogen metabolites to macromolecules and, in addition, the relationship of these parameters to the occurrence of pulmonary neoplasia resulting from exposure to polycyclic hydrocarbon carcinogens.

Date Contract Initiated: June 23, 1973

Current Annual Level: $146,705

MISSOURI, UNIVERSITY OF (NO1-CP-33335)

Title: Effect of a High-Meat Diet on the Bacterial Flora and Chemical Components of Feces

Contractor's Project Director: Dr. David J. Hentges

Project Officer (NCI): Dr. Carl E. Smith

Objective: To determine if high-meat diet alters the bowel flora and the chemical composition of feces of humans.

Major Findings: Ten volunteers completed a four-month diet series consisting of a control diet, a meatless diet, a high meat diet, and a second control diet, each of one month duration. Fat and fiber contents were essentially the same in all four diets, but protein content was doubled during the high meat diet. During the fourth week on each diet, three stool specimens collected from each volunteer were analyzed for aerobic and anaerobic bacteria, proximates and minerals, individual amino acids, creatine and creatinine, tryptophan metabolites, polyamines, fatty acids, and neutral and acid steroids.

High beef protein consumption had little effect on the composition of the intestinal flora. There were no significant differences in total counts of

anaerobic or facultative and aerobic organisms in the feces when volunteers were on meatless or high beef diets. There was some increase in the proportion of anaerobic to aerobic bacteria in the feces, however, during the high meat diet as compared with the meatless diet. There was no increase in the concentrations of either neutral or acid steroids. Although the high meat diet contained more cholesterol than the meatless diet, less cholesterol and cholesterol metabolites were excreted during high meat consumption. Two of the volunteers excreted significantly greater quantities of cholesterol than the other eight volunteers, irrespective of diet.

Significance to Biomedical Research and the Program of the Institute: Epidemiological studies indicate that a relationship exists between the incidence of colon cancer and the quantity of meat consumed by a population. A systematic study of the effects of a high-meat diet on the composition of the intestinal contents of humans had never before been undertaken. Results provide information on changes that occur in the fecal flora and in the concentrations of potentially carcinogenic compounds present when individuals are placed on a high-meat protein diet. This information is essential to an understanding of the role of diet in the etiology of colon cancer.

Proposed Course: The contract expired December 5, 1976

Date Contract Initiated: June 5, 1973

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (at Lincoln) (NO1-CP-33289)

Title: Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture

Contractor's Project Director: Dr. Mihir R. Banerjee

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To assess whether chemical carcinogen-induced, nodule-like alveolar lesions (NLAL) and/or other latent transformed cells may possess any irreversible abnormal characteristics including tumorigenicity.

Major Findings: The whole mammary gland of 3-4 week old female mouse is induced to pregnancy-like alveolar development in a chemically defined culture medium containing the hormones insulin (I), prolactin (Prl), aldosterone (A), and cortisol (F). Subsequent cultivation of the gland in a hormone-deficient medium (I + A) results in alveolar regression, leaving mainly a ductal parenchyma. Mammary glands cultivated for 9 days in culture are treated with dimethylbenzanthracene (DMBA), 2 μg/ml, for a 24-hour period at different times during hormone-induced alveolar growth. The alveoli are then allowed to regress in medium with I + A. Nodule-like alveolar lesions (NLAL) are present in 80% of the DMBA-treated glands

cultivated with IPrlAF. The carcinogen treatment between the third and fourth days of cultivation is most effective, and dimethylsulfoxide-treated gland failed to show any NLAL. The cytotoxicity of DMBA is pronounced when glands are treated with the hydrocarbon at 24 and 48 hr. of culture, and these glands fail to produce alveolar development during 9 day culture. Equimolor concentrations of less potent hydrocarbons, dibenzanthracene and benzanthracene, and of anthracene under similar conditions of cultivation so far have been low in their NLAL-inducing action. The results of another study also show that ^{3}H-DMBA binds to DNA of mammary cells in culture, and spectral analysis of DNA indicates a detectable level of binding of the hydrocarbon as measured by absorbance at 310 nm, suggesting that the hydrocarbon nucleus covalently binds to mammary DNA. These findings indicate that the oncogenic compound, DMBA, undergoes metabolic activation and behaves in a conventional manner in the present culture system.

Significance to Biomedical Research and the Program of the Institute: The findings thus far appear encouraging with regard to potential of the present organ culture system to develop into a bioassay for monitoring the action of oncogenic chemicals in breast carcinogenesis.

Proposed Course: While results thus far indicate that the more potent carcinogenic compound DMBA is most active, assessment for potential tumorigenicity, other associated pathology of NLAL, and/or latent transformed cells must be done by transplantation in vivo. The primary emphasis of the proposed course of the research period will be to transplant these tissues into gland-free mammary fat pads of virgin isogenic host mice to assess the tumorigenic potential of NLAL and/or latent transformed cells in DMBA-treated glands.

Date Contract Initiated: June 1, 1973

Current Annual Level: $66,622

NEW YORK UNIVERSITY (NO1-CP-33260)

Title: Studies in Pulmonary Carcinogenesis

Contractor's Project Directors: Dr. Marvin Kuschner
Dr. Sidney Laskin

Project Officer (NCI): Dr. Carl Smith

Objectives: The objectives of this contract have been the investigation of the mechanisms of pulmonary carcinogenesis and the examination of the carcinogenic potential of suspected materials. Its aims are to illuminate the process of malignant change as exemplified in the lung while, at the same time, providing immediate means for the prevention of lung cancer by the identification of hazardous materials. In particular, since it has become more and more evident that the induction of human lung cancer

is multi-factorial in nature and dependent upon long-term, low level exposures to combined agents, one objective of this contract is to evaluate this combined action in a wide variety of animal models using a variety of experimental techniques.

Major Findings: Animal models have been developed which duplicate human bronchogenic carcinoma. The production of such tumors has been achieved by a variety of techniques which include thread transfixion, pellet inplantation, direct intrabronchial instillation, and finally, inhalation. Dose-response relationships have been developed from the intrabronchial pellet technique with polycyclic hydrocarbons.

An extensive series of studies are being performed in this laboratory to study the effects of several components of air pollution. This laboratory was the first to produce lung cancer in rats with combined inhalation exposures to sulfur dioxide and benzo(a)pyrene. Studies with combined carcinogen-irritant exposures were performed in an effort to reinforce the original results. Findings confirmed the original studies.

In order to define the progression of changes which result in cancer, an extensive dose-response study using hamsters and intratracheal intubations of methylcholanthrene was performed. Results showed an excellent dose-response relationship, a shortening of the time needed to develop cancer as dose increases, and cancer incidence approaching 100% at 24 intubations of 1 mg methylcholanthrene.

The hamster intubation technique has also provided a valuable model for carcinogen-irritant studies. Animals intubated with carcinogens were exposed by inhalation to sulfur dioxide atmospheres. Results have shown findings of adenocarcinoma in animals given 10 intubations of methylcholanthrene and lifetime exposures to sulfur dioxide. Benzo(a)pyrene, which as shown negative results by intubation only, in combination with exposures to sulfur dioxide has shown both squamous cell carcinomas and adenocarcinomas in the lung.

Short-term and chronic inhalation studies with nitrogen dioxide, a component of air pollution and cigarette smoke, are complete. Although mucosal changes after exposures to 25 ppm appear to be minimal, endothelial damage has been reported with electron microscopy. Later results suggest a low incidence of panacinar emphysema, particularly in the hamster.

Studies have been initiated for combined exposures of rats and hamsters to nitrogen dioxide and benzo(a)pyrene. Results, to date, have shown findings of 2 squamous cell carcinomas in rats exposed to nitrogen dioxide and benzo(a)pyrene for 1 hour per day. One rat exposed to nitrogen dioxide for 6 hours followed by a 1-hour exposure to nitrogen dioxide plus benzo(a)-pyrene showed an angiosarcoma with multiple metastases to the lung.

Preliminary inhalation studies with croton oil have been completed. Studies involving carcinogen intubations with subsequent croton oil inhalation exposures are underway. Techniques employed involve the use of single or

multiple intubations in hamsters with methylcholanthrene or benzo(a)pyrene followed by inhalation of croton oil. Results, to date, show an increase in cancer incidence in the groups exposed to benzo(a)pyrene or methylcholanthrene followed by croton oil when compared to those receiving the carcinogen alone.

Compounds of chromium have been demonstrated to be capable of producing bronchogenic carcinoma of the lung in experimental animals. The identification of calcium chromate as one of the major agents in the chromate problem has resulted in inhalation and clearance studies with this compound. The recently completed chronic study at 2 mg/cu m has produced carcinomas in rats and hamsters.

Studies are underway with current industrial chrome process materials provided by the industry. Since a survey of the industry and evaluation of experimental results implicates process residue as the major potential agent, a chronic inhalation study at 20 mg/cu m process residue has been performed. Two of the 78 rats in this study developed respiratory tract tumors. This is an incidence entirely comparable to that seen with pure calcium chromate aerosol so that there appears to be no contribution to the activity of this compound by other materials in the residue.

Two industrial plastic dusts were studied to determine their possible effects on workers in the industry. Reinforced polyester-fiberglass plant dust was studied utilizing the intubation technique and short-term inhalation exposures. Centrilobular emphysema and other suggestive changes of interest were noted. With polyurethane foam, animals were given 30 inhalation exposures to freshly generated foam dust atmospheres at 2 levels. Centrilobular emphysema and squamous cell carcinoma were seen in rats at both levels. In view of the large population of construction workers involved in the use of such materials, chronic studies are being developed for the polyurethanes.

Inhalation studies were completed with two chlorinated ethers used widely as industrial reaction intermediates. Both compounds proved to be carcinogens. The bis(chloromethyl)ether showed extremely high incidences of carcinoma at 0.1 ppm. The extremely low levels and the magnitude of the carcinoma response with bis(chloromethyl)ether suggested a very serious new industrial hazard. This has been reinforced by the publication of several reports documenting human cases in exposed workers. These compounds are now listed in the OSHA carcinogen regulations. Preliminary studies in this laboratory suggest that hydrochloric acid and formaldehyde, at levels approximating industrial exposures, can produce bis(chloromethyl)ether. The widespread use of such materials indicates the need for animal inhalation studies.

Results with chloromethyl methyl ether and bis(chloromethyl)ether have prompted examination of a variety of other alkylating agents of industrial importance.

In short-term exposures to 1 and 3 ppm dimethylcarbamoyl chloride, several tumors of the nasal cavity were seen. Four squamous cell carcinomas of the

trachea were seen in a group receiving 15 exposures to 10 ppm. In a lifetime exposure at 1 ppm, 94 of 98 rats were seen with squamous cell carcinomas of the nasal cavity. These appeared as early as 196 days with a mean cancer time of 261 days. These results suggest a new industrial hazard of extreme importance.

Epichlorohydrin, another widely used industrial intermediate, is currently under test. Range-finding studies have been completed including LC_{50}'s and thirty-day pilot studies. Chronic studies with rats at 30 and 10 ppm are in progress. In the course of the range-finding studies, 40 rats were exposed to 30 daily exposures of 6 hours each to 100 ppm epichlorohydrin. Twenty-eight of the 40 rats involved have died. In these 28, three animals dying at 460, 490, and 596 days, respectively, had squamous cell carcinomas of the nasal epithelium. A fourth animal, dying at 391 days, had a squamous cell papilloma. Two of the 12 animals still alive in the 100 ppm group have developed nose masses in a manner akin to those observed during life in animals with later histologically confirmed cancer. Additional experiments started later include exposures of groups of rats at concentrations of 100, 30, and 10 ppm epichlorohydrin. An early warning report of the potential hazard of this material has been submitted to the concerned Federal agencies, the director of the NCI Clearinghouse on Environmental Carcinogens, industries, and the unions.

The inability to demonstrate the carcinogenicity of arsenic in experimental animals has led to the suspicion that its tumor-producing properties in humans may depend upon exposures combined with other substances. The particular combination of arsenic and sulfur dioxide to which smelter workers are exposed is one to which Contractor's previously successful benzo(a)pyrene + sulfur dioxide system would lend itself well. Accordingly, extensive preliminary technical development has been undertaken, and Contractor is about to begin a series of combined sulfur dioxide + arsenic trioxide range-finding exposures with a view to establishing a tolerated dose for chronic exposure.

Significance to Biomedical Research and the Program of the Institute: This contract represents a component of a broad effort to develop bioassay techniques for studies on lung cancer. These have and will continue to be of importance in identifying potentially hazardous materials to which the working population is exposed. This role in preventive medicine has and will be expected to significantly reduce industrial cancer cases.

Proposed Course: To extend previous studies and to develop inhalation studies with other types of suspected materials.

Date Contract Initiated: January 1, 1973

Current Annual Level: $521,071

NORTHWESTERN UNIVERSITY (NO1-CP-75876) [Formerly University of Kansas (NO1-CP-23271)]

Title: Histogenesis of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Janardan K. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: This project was initiated to confirm earlier reports of induction of pancreatic adenocarcinoma in guinea pigs by N-methyl-N-nitrosourea (MNU) and to study the histogenesis of pancreatic tumors. An additional objective of this project is to manipulate this model so as to reduce the induction period and increase the incidence of pancreatic adenocarcinoma.

Major Findings: A number of agents capable of inducing acute and chronic changes in exocrine pancreas of the guinea pig were identified. These include: lasiocarpine, 4-hydroxyaminoquinoline-1-oxide, cobaltous chloride, aflatoxin B_1, and azaserine.

By using a single intravenous injection of 4-hydroxaminoquinoline-1-oxide, a model to induce pancreatic necrosis and regeneration in strain 13 inbred guinea pigs was developed. This model of pancreatic regeneration should be useful to study the potentiating effect of MNU on the incidence of pancreatic tumors when administered during peak regeneration.

Pancreatic adenocarcinomas developed in 10 of 34 inbred strain 13 guinea pigs between 28-44 weeks of MNU treatment. In other animals atypical changes involving acinar cells were observed in some pancreatic lobules. These changes are suggestive of acinar cell dedifferentiation and their role, if any, in the histogenesis of pancreatic adenocarcinoma remains to be elucidated. These studies suggest the feasibility of using inbred guinea pigs for developing a satisfactory model of pancreatic adenocarcinoma. Additional studies are necessary to minimize the high mortality rate (54%) during the first six months and to enhance the incidence of pancreatic adenocarcinoma.

The effect of long-term administration of azaserine and 2,2-dihydroxy-di-n-propylnitrosamine (DHPN) on pancreas in inbred strain 2 guinea pigs was investigated. Hyperplastic nodules or carcinomas of the pancreas were not seen in animals treated with azaserine for 24 weeks at a dose level of 5 mg/kg b.wt., twice weekly. DHPN at a dose of 250 mg/kg body weight/week for 30 weeks, produced angiosarcomas of the liver in 23 of 32 guinea pigs, between 22 and 40 weeks. Acinar cell adenoma of the pancreas was noted in one animal. In another animal acinar cell carcinoma of the pancreas was found.

Significance to Biomedical Research and the Program of the Institute: The incidence of pancreatic adenocarcinoma in this country is progressively increasing. Development of animal models of pancreatic carcinoma is most

important to the program. Further development and refinement of the guinea pig MNU model is one of the major goals of the Program; this project, along with others, is designed specifically to achieve this goal.

Proposed Course: Additional studies, using large numbers of inbred strain 13 guinea pigs, will be undertaken to confirm the rapid development of pancreatic tumors in this inbred strain, using MNU. Sequential morphologic studies to determine the histogenesis, particularly the role of acinar cell dedifferentiation, and progression of this cancer will be continued.

Date Contract Initiated: January 3, 1977

Current Annual Level: $43,148

ORGANIZATION FOR HEALTH RESEARCH (TNO) (NO1-CP-33330)

Title: Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. J. J. Broerse

Project Officers (NCI): Dr. D. Jane Taylor
Dr. Carl E. Smith

Objectives: The objectives in these studies include: a) investigation of the nature of the dose-effect relationships for x-rays and neutrons which might contribute to the insight into the mechanism of radiation carcinogenesis, b) study of the synergistic interaction of hormone administration and neutron irradiation, and c) determination of the relative biological effectiveness (RBE) of neutron beams with three different energies to assess the relative risk of neutron irradiation. Three rat strains of expected different susceptibilities to induction of mammary tumors to ionizing radiation are employed. A further objective is elucidation of causes of such susceptibility differences.

Major Findings: For all three neutron energies the dosimetric measurements have been completed, and the irradiation conditions for the neutrons and the x-rays have been designed to arrive at directly comparable exposure conditions.

Breeding colonies of Sprague-Dawley (SD), Brown Norway (BN), and Wister (WAG) rats have been established and are meeting the requirements of the study. All groups of intact as well as sterilized females and sterilized females treated with hormone have been entered into the study and exposed to the designed x- and neutron-irradiation, bringing the number of animals in the program up to a total of 5,000.

Studies on the optimal method of hormone administration have been completed. The natural hormone estradiol-17-beta (E-2) is used in almost all studies. Fused E-2 pellets in paraffin-cholesterol provide a more constant release

than E-2 pellets pressed or fused with cholesterol as a single carrier. Ethynyl estradiol release from paraffin-cholesterol pellets did not differ from that of E-2.

Preliminary results on mammary tumorigenesis in the intact female rats are now available. At the present time, results are based largely on gross morphology; histological distinction between benign and malignant tumors has been made only in part. In the non-irradiated control groups, first mammary tumors are only observed at an age between 19 and 23 months. This is a latent period considerably longer than described in other studies. Moreover, these non-irradiated controls show differences in susceptibility to mammary tumorigenesis: at approximately 32 months mammary tumor incidences are 88, 39, and 9% for Sprague-Dawley (SD), WAG/Rij and Brown Norway (BN) rats, respectively, confirming initial predictions of susceptibility differences. Non-irradiated control hystero-ovariectomized animals have tumor incidences considerably lower than the intact control animal groups.

In the irradiated groups, cumulative tumor incidences must be scored at a time when the number of spontaneously occurring tumors is still very low. If such comparisons are made at 20 months, susceptibility to mammary tumorigenesis after x-irradiation is not greatly different for WAG/Rij and BN rats. However, for 15 MeV neutron irradiation, WAG/Rij rats show clearly an intermediate susceptibility between that of SD and BN animals. For both x-ray and 15 MeV neutron irradiation, SD rats are most susceptible.

Morphological data has been obtained from 272 rats of control, x-ray, and 15 MeV neutron irradiated groups. The occurrence of benign and histologically malignant mammary gland tumors for the three rat strains has been determined. These very preliminary observations, for these irradiation groups, indicate that in all three rat strains irradiation causes a substantial increase in benign tumors while not increasing at all or only slightly the incidence of carcinoma.

Significance to Biomedical Research and the Program of the Institute: The Program is expected to provide an insight into the interaction of estrogen levels and radiation carcinogenesis in the mammary gland which is of relevance to the problem of human breast cancer.

Proposed Course: In the groups of estrogen-treated animals the first tumors have been observed. In the coming period, a start will be made with the scoring of tumor incidence for parallel groups of animals which were hystero-ovariectomized and/or estrogen-treated. All relevant data of animals included in the study are presently stored on magnetic tape and will be processed by a small digital computer in order to enable direct retrieval of the status of the project with regard to latency period, single or multiple occurrence of tumors, histological type of tumors, and dose-effect relationships.

As more histological data become available, an attempt will be made to correlate the various histological types of mammary tumors with the type and dose of radiation, with the presence or absence of exogeneous hormones,

and with the effect of hystero-ovariectomy. The histological classification of mammary gland lesions will include: 1) lobular hyperplasia, 2) adenoma, 3) fibroadenoma, 4) fibroma, 5) adenocarcinoma, 6) papillary carcinoma, 7) anaplastic carcinoma, 8) cribiform carcinoma, 9) squamous cell carcinoma, and 10) sarcoma.

Date Contract Initiated: June 25, 1973

Current Annual Level: 0

OXFORD, UNIVERSITY OF (NO1-CP-23215)

Title: Procurement of Data for Determination of Disease Linkage

Contractor's Project Director: Dr. John H. Baldwin

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To investigate hypothesis that certain diseases (such as coronary heart disease) are caused by the same factors as specified bowel tumors, and will, therefore, be linked in individuals in a general population more frequently than would be expected by chance.

Major Findings: This project uses systems of linked medical records for ascertainment of spatial or temporal associations between certain diseases and colon or rectal cancers. It has also been collecting and analyzing data on the geographic distribution, sex, age, and social class dependencies of colon and rectal cancers in England, specifically within the Oxford Record Linkage Study area. The methods of analysis of the data have themselves been subject to scrutiny and test, and revised methods of greater refinement have been devised and employed.

Searchers for such disease links in people have found statistically significant associations between: a) colon and rectal cancers; b) cancer of the colon and cancer of the breast in women; c) cancer of the colon and cancer of the prostate in men; d) rectal cancers and a group of genital cancers; e) large bowel cancers and benign polypi, a known association which was quantified; and f) ulcerative colitis and subsequent colon cancer, another known association which was strongly confirmed in women.

Analysis also indicates that: a) diverticulitis, though statistically associated with large bowel cancer, seems unlikely to be a participant in carcinogenesis; b) appendicitis may occur more often than expected in colon cancer patients; and c) no associations are found with arteriosclerotic heart disease.

It is important to realize that the analyzes to the present time are based on a five-year period (1963-1967) only. Consequently, the very short

period of follow-up (mean 2.5 years for Oxfordshire patients and 1 year for West Berkshire patients) entails that any diagnostic association requiring more than about 1.5 years to become manifest is unlikely to be demonstrable at the present time. This emphasizes the importance of extending the time span of linked record systems.

Significance to Biomedical Research and the Program of the Institute: The association of colon cancer and other diseases in populations has been the subject of much discussion. Determination of the links in individuals would provide invaluable clues to etiology and, as well, provide markers of high risk for detection programs. While this kind of information is sought from U.S. sources, all are small and/or highly selective populations, and the data are definitely not readily accessible. Major segments of later parts of the program will be based on these epidemiological findings.

Proposed Course: This contract expired on December 31, 1976

Date Contract Initiated: April 9, 1972

Current Annual Level: 0

PASADENA FOUNDATION FOR MEDICAL RESEARCH (NO1-CP-65850)

Title: Primary Culture of Normal, Human Prostatic Epithelial Cells

Contractor's Project Director: Dr. M. Edward Kaighn

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To develop procedures for establishment and characterization of primary cultures of normal, human prostatic epithelial cells.

Major Findings: This is a new contract. As yet, no major findings can be reported.

Significance to Biomedical Research and the Program of the Institute: Successful cultivation of normal, human prostatic epithelial cells will provide a valuable system for carcinogenic studies. Defining the nutritional requirements for replication and for maintenance of specific functions of these cells will furnish important clues regarding the control mechanisms of the normal cell and how these differ in their malignant counterpart.

Proposed Course: 1) Optimum methodologies will be developed for the primary dissociation of the normal prostate. 2) Homogenous populations of epithelial cells will be derived using the clonal isolation method. 3) Enzymatic and chemical methods will be evaluated to facilitate subculturing of the epithelial cell clones. 4) By use of the clonal assay procedure, a low serum (defined) medium will be developed which supports optimal growth of the epithelial cells. 5) The normality of the cultured epithelial cells will be ascertained using karyology, histochemical analysis, and electron microscopy.

Date Contract Initiated: September 30, 1976

Current Annual Level: $101,052

SOUTHERN RESEARCH INSTITUTE (NO1-CP-22064)

Title: Organ Culture Assay of Vitamin A Analogs

Contractor's Project Directors: Dr. Lee Wilkoff
Dr. Donald Hill

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The objectives of this contract are: 1) to develop adequate bioassays to evaluate the biological activity of new retinoids (vitamin A analogs) as measured by control of epithelial differentiation; 2) to perform bioassays on selected analogs; 3) to develop new quantitative assays for retinoid activity in organ culture or tissue culture; 4) to evaluate the morphological, histological, and biochemical aspects of toxicity and pharmacology of selected retinoids to rodents; and 5) to investigate the biochemical changes caused by carcinogens in target tissues and their possible reversal by retinoids.

Major Findings: The methylketo and 1-methoxyethyl analogs of retinoic acid were at least 50-fold more active than retinoic acid in reversing N-methyl-N'-nitro-N-nitrosoguanidine-induced hyperplasia of mouse prostate explants. The trimethylmethoxyphenyl analog of retinoic acid and retinyl methyl ether were significantly more active than retinoic acid in this system. N- Acetyl-retinylamine, retinal acetyl hydrazone, and retinal oxime were as active as retinoic acid.

Selected retinoids also were evaluated for their effect in reversing benzo(a) pyrene-induced hyperplasia in mouse prostate explants. The proliferative response of the prostatic epithelium to benzo(a)pyrene was very actively reversed by 13-cis-retinoic acid. The following five analogs were at least as active as retinoic acid: the ethyl ester of the trimethylthiophere analog of retinoic acid, the 10-fluoro analog of all-trans-trimethylmethoxyphenyl methyl retinoate, the 10-fluoro analog of 13-cis-trimethylmethoxyphenyl methyl retinoate, the 12(Z)-fluoro analog of ethyl retinoate, and the 12(E)-fluoro analog of ethyl retinoate.

Retinoic acid reversed testosterone-induced hyperplasia in the epithelium of mouse prostate explants.

Organ culture of mouse prostate may be employed as a test system to obtain preliminary information regarding the potential carcinogenicity of a compound. Hyperplasia of prostatic epithelium was induced after treating for 8 days with 3-methylcholanthrene, benzo(a)pyrene, the epoxide of methylcholanthrene, or

N-methyl-N'-nitro-N-nitrosoguanidine. Noncarcinogenic compounds such as pyrene and phenanthrene did not produce any mitotic stimulatory effect. A retinoic acid-binding protein (RABP), which may mediate the function of retinoic acid by serving as its cellular receptor, is present in various epithelial tissues of animals. The protein is readily detectable in brain, skin, testis, and eye of these animals and is present in smaller quantities in the bladder, prostate, uterus, trachea, and mammary glands of rats and mice. Relatively larger amounts of the protein are present in the tissues of young animals as compared to older ones. Although RABP cannot be detected in normal lungs, it is present in the transplantable Lewis lung tumor of mice and in lungs with metastatic Lewis lung foci. Of four transplantable colon tumors of mice, two contain RABP. RABP binds specifically retinoic acid and its analogs having a free carboxyl group and a sidechain not drastically altered. None of a variety of vitamins, hormones, and cofactors greatly affected the binding of retinoic acid to RABP.

Although the binding affinity of RABP for retinoic acid and its synthetic analogs correlates well with their biological activity, no such correlation can be established for their binding to serum albumin, the protein involved in transport of retinoic acid and its analogs.

Experiments determining the subacute lethality of retinoic acid and 13-cis-retinoic acid have confirmed a previous report that the 13-cis analog is less toxic to mice. The LD_{50} for retinoic acid is 31 mg/kg and 1100 mg/kg for doses given i.p. and p.o., respectively, for 21 days. Comparable values for 13-cis-retinoic acid are 140 and 26,000 mg/kg. The 13-cis compound causes the same number of bone fractures at 3-5 times the dose of retinoic acid. Treatment of mice with either compound given i.p. results in a dose-related decrease in the hemoglobin concentration and packed cell volume. A dose-related decrease in the erythrocyte count was caused by 13-cis-retinoic acid but not by retinoic acid.

A method employing trifluoroacetic acid for the quantitative determination of retinoid acids in biological media has been developed. As measured by this method, the amounts of 13-cis-retinoic acid that appear in the serum of mice, rats, and hamsters were greater than those of retinoic acid, suggesting that the toxicity is due to factors other than absorption into the blood. 13-cis-retinoic acid binds more tightly to human serum albumin than retinoic acid, a factor possibly involved in the lower toxicity of the 13-cis compound. Tight binding to serum albumin could prevent the diffusion of 13-cis-retinoic acid into cells.

Significance to Biomedical Research and the Program of the Institute:
Vitamin A is required for the normal differentiation and growth of the epithelium in the following organs, all of which are major sites of primary cancer in man: bronchi and trachea, colon, stomach, uterus, kidney and bladder, testis, and skin. The role of vitamin A and other retinoids in controlling normal differentiation and growth in tracheobronchial and intestinal epithelium is currently under investigation in the lung cancer program of the NCI.

In addition to controlling the differentiation of many types of normal epithelium, vitamin A and other retinoid compounds are also able to alter the process of chemical carcinogenesis in several different epithelia and to block the effects of several different carcinogens. Thus, retinoids can block the carcinogenic effects of polycyclic hydrocarbons on tracheobronchial, gastric, and uterine (cervical) epithelium. In some of these studies, it has been shown that the effect of vitamin A may not be due to alterations of the immediate metabolism of the carcinogen, since vitamin A is able to inhibit carcinogenesis even when administered several weeks after the carcinogen. Studies are current in progress in the lung cancer program to elucidate the role of retinoids in blocking epithelial carcinogenesis. Presumably this effect is directly related to the biochemical and cellular role of retinoids in determining normal differentiation in these tissues, but the proof of this is not yet at hand.

The ultimate implications of these studies on the inhibition of epithelial carcinogenesis are very important. If vitamin A is indeed functioning as a differentiation hormone, it should be possible, with the cooperation of organic chemists, to design and synthesize new retinoids having even greater potency than vitamin A on differentiation of epithelia. The precedents in steroid chemistry for this approach to vitamin A are impressive; presently there are many synthetic steroid analogs that are more potent (and often more specific and less toxic) than the naturally occurring estrogens, progestational agents, androgens, or adrenocortical steroids. If this type of approach can be applied to the hormonal action of vitamin A, the potential for inhibiting carcinogenesis in many types of epithelia would be greatly increased.

Proposed Course: Continue the development of bioassay methods using organ culture systems to evaluate the biological activity of retinoids. Devise new methods, using morphological, histochemical, and biochemical end points for quantitative bioassay of retinoids. Determine the biochemical characteristics and biological role of retinoic acid-binding protein, and attempt the development of a biochemical screen for retinoid activity using this protein. Continue the evaluation of the morphological, histological, and biochemical aspects of toxicity and pharmacology of selected retinoids in rodents. Study the biochemical changes caused by carcinogens in target tissues and their possible reversal by retinoids.

Date Contract Initiated: December 10, 1971

Current Annual Level: $421,057

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-33379)

Title: Studies of Hormonal Factors of the Human and Animal Prostate

Contractor's Project Director: Dr. Sydney A. Shain

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The relationship between aging-associated changes in endocrine regulation of prostate function and the pathogenesis of prostate adenocarcinoma is ill-defined and poorly understood. The principal objectives of this contract have been the interage and interspecific evaluation of prostate androgen metabolism and the cellular content, distribution, and specificity of prostate androgen receptors in normal, lesion-free senile and neoplastic specimens of rodent, canine, and human prostate. Spontaneous adenocarcinomas of the AxC rat ventral prostate were detected with a frequency of 70 percent in rats greater than 30 months of age. The primary lesions were transplantable to isogenic hosts.

Major Findings: Androgen receptors of the prostate of the aging beagle: Methodology was developed for the determination of cytoplasmic and nuclear androgen receptors of canine prostate obtained from intact donors.

Determination of cytoplasmic and nuclear androgen receptor content of prostate of aging beagles demonstrated the total androgen receptor content of aged beagle prostate was 4.7-fold greater than that of young beagles; the consequence of an aging-related proliferation of receptor-containing cells as demonstrated by the fact that total DNA, RNA, or protein content of the prostate of the aged beagle was approximately 4.7-fold greater than that of the young beagle.

This result is in complete opposition to that which pertains in the prostate of the aged rat. In this species, significant aging-associated decreases in ventral prostate cytoplasmic and nuclear androgen receptor content per cell and dorsolateral prostate nuclear receptor content per cell have previously been demonstrated by Contractor.

Androgen receptors of the human prostate: Multiple determinations of available cytoplasmic androgen receptor content of cytosol from human prostate obtained either by transurethral resection (TURP) or by suprapubic or retrapubic prostatectomy have been completed.

Steroid specificity of human prostate cytoplasmic androgen receptor was comparable to that of rat or canine prostate cytoplasmic receptor. The concentration of total cytoplasmic androgen receptor in human prostate cytosol was identical to the concentration of total cytoplasmic androgen receptor sites found in prostate from young intact dogs.

The concentration of the human prostate nuclear androgen receptor was approximately sevenfold less than that of the comparable canine prostate receptor.

Testosterone metabolism by human prostate specimens: When human benign prostate hyperplasia (BPH) obtained by prostate enucleation was incubated with testosterone at 1.5×10^{-7} M, the principal metabolite produced was 5α-dihydrotestosterone, which accounted for 80 to 93 percent of the metabolic products. The inclusion of estradiol, 1.5×10^{-7} M, in these incubations decreased 5α-dihydrotestosterone production by 10 percent.

When the same hyperplastic prostate specimens were incubated with testosterone, 10^{-6} M, a profound shift to less reductive metabolism of testosterone was demonstrated. Under these experimental conditions, 5α-dihydrotestosterone represented 36 to 59 percent of the metabolites elaborated. The inclusion of estradiol, 10^{-6} M, decreased 5α-dihydrotestosterone production 35 percent. These experiments indicate the principal effect of estradiol was inhibition of human prostate Δ^4-3-ketosteroid 5α-oxidoreductase activity in the absence of any effect upon 17β-hydroxysteroid dehydrogenase activity.

Testosterone metabolic capacity of human benign hyperplastic prostate obtained by transurethral resection (TURP) was more heterogeneous than that of the enucleation specimens, but the data indicate the absence of a significant difference in the testosterone metabolic capacity of human benign hyperplastic prostate obtained by these two surgical protocols.

Preliminary examination of testosterone metabolic capacity of human prostate adenocarcinoma obtained by TURP demonstrated this tissue to have a diminished capacity to produce 5α-dihydrotestosterone and increased capacity to produce Δ^4-androstenedione. These preliminary data suggest a predominant feature of human prostate adenocarcinoma may be diminished capacity to produce 5α-dihydrotestosterone and increased capacity to produce Δ^4-androstenedione from testosterone.

Testosterone metabolism by the prostate of the aging canine: Continuing evaluation of testosterone metabolism by the aging canine prostate confirms Contractor's earlier reported observation of an aging-associated shift to increasingly reductive testosterone metabolism.

Transplantable adenocarcinomas of the AxC rat ventral prostate: Two primary spontaneous adenocarcinomas of the AxC rat ventral prostate have yielded three subcutaneously transplantable adenocarcinomas which have retained many of the histologic features of the original spontaneous tumors. On transplant the tumors grow in the subcutis as large hemorrhagic lesions. The tumors consist of neoplastic cells with pleomorphic nuclei and finely clumped chromatin and are organized in typical cribriform pattern. The transplantable tumors are slow-growing adenocarcinomas, and one second-passage tumor has been biopsied and transplanted to third-passage recipients.

Preliminary examination of testosterone metabolic potential of the transplantable adenocarcinomas has demonstrated markedly oxidative testosterone metabolism, reminiscent of that seen in human prostate adenocarcinoma.

The development of precocious prostatic senility in the AxC rat as a model for the evaluation of promoters of prostate carcinogenesis: These studies are evaluating the utility of medrogestone and melatonin as antigonadal agents for the controlled decrease of plasma testosterone concentration in AxC rats in the absence of profound involution of the ventral prostate. Rats are currently being entered into Stage II evaluations to define more precisely the appropriate dosage of medrogestone and melatonin which will achieve modest prostate involution concurrent with significantly diminished

plasma testosterone concentration. Subsequent to the identification of the desired dosages of the anti-gonadal agents, rats will be entered into long-term experiments in which the development of prostatic senility will be monitored by previously documented aging-associated changes in testosterone metabolic potential and prostate androgen receptor content and distribution.

Significance to Biomedical Research and the Program of the Institute: Contractor's studies of aging-associated changes in biochemical and endocrinologic processes associated with prostate homeostasis have identified specific alterations which appear to be related to prostate pathology. The elucidation of these alterations in prostate homeostatis which may be related to the pathogenesis of prostatic adenocarcinoma will facilitate the determination of possible causes of and provide for the development of methodology for early detection of risk of prostate adenocarcinoma. In addition, identification of causative factors will allow for rational endocrine manipulation in an attempt to reduce the incidence, mortality, and morbidity of prostate cancer.

Proposed Course: This project will continue to evaluate specific changes in hormonal regulation of prostatic epithelial cell function during aging. In addition, lesion-free and lesion-containing prostate tissue will be characterized for alterations in intercellular regulators of cell function. Transplantable adenocarcinomas of the ventral prostate will be established as slow-growing and rapidly-growing tumors as models for the study of the progression of prostate cancer. Finally, Contractor's model for precocious prostatic senility will be developed as a means of studying possible promoting factors associated with neoplastic transformation of prostatic epithelial cells.

Date Contract Initiated: May 31, 1973

Current Annual Level: $ 351,649

STATE UNIVERSITY OF NEW YORK (at Stony Brook) (NO1-CP-33361)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. Bernard P. Lane

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: 1) To determine the methodology for testing for biologic (in vivo) malignancy of tracheal epithelium transformed in organ culture, 2) to determine the optimal conditions for the organ culture of tracheal rings, 3) to observe the biology of tracheal ring cultures, 4) to determine the effects of exposing tracheal rings to the carcinogen benzo(a)pyrene (BP) in vitro, and 5) to monitor cultures exposed in vitro for malignant transformation.

Major Findings: 1) Methods for large-scale, long-term culture of rat tracheal tissues were developed during past contract periods. McCoy's 5a (modified) medium buffered to pH 6.8-7.3 with sodium bicarbonate and maintained in an atmosphere of 5% CO_2 with air will support growth. Using a mechanical slicing device, cultures are prepared in batches of 300-500 and grown under these conditions for periods of months. The cultures are uniform in appearance and in the ability to respond to various stimuli by cellular repair and by mitotic activity. Studies of epithelial growth and maintenance of differentiation in CMRL 1066, Parker's 199, and Dulbecco's modification of Eagle's MEM demonstrated that these alternative culture media offer no advantage over McCoy's 5a (modified) medium. A study of the role of serum in epithelization of the free-floating culture, using light, transmission electron and scanning electron microscopy, established the basis for observed differential patterns of the appearance of ciliated cells and dividing cells on the surfaces of the culture. Serum was shown to facilitate migration of the epithelial cells as a continuous flattened sheet.

2) A series of protocols for exposure of the cultures to carcinogens was carried out. Epithelium was shown to be exquisitely sensitive to BP if the culture medium is free of serum. Continuous exposure to BP in the culture medium was found to result in shortening of the life of the culture in rough proportion to the concentration of this carcinogen. A maximal effect, consisting of hyperplasia, after two weeks of exposure to 2 ugm/ml BP gives way to atrophy if exposure is continued for several weeks longer. To avoid the apparent masking of carcinogenicity by cytotoxicity of the agent, two modified exposure regiments were evaluated. When 30-hour exposure periods were alternated with 72- to 138-hour periods in medium lacking the carcinogen, cell death occurred as observed with continuous exposure. However, cultures exposed continuously for two weeks to 2 ugm/ml and then removed to carcinogen-free medium developed areas of focal hyperplasia, which may be equivalent to nodular hyperplasias in livers of animals treated with carcinogens. They persist for six or more weeks and are reversed or prevented from appearing by addition to the medium of retinyl acetate, retinoic acid, and three of four synthetic analogues of retinoic acid tested. The mechanism underlying this reversion is being studied. The cells of carcinogen-induced hyperplastic regions have also been shown to secrete plasminogen activator and to have abnormal plasma membrane structure.

3) Previous work established that Wistar Lewis rats were suitable as both donor and host for studies involving implantation of carcinogen-treated organ cultures into intact animals to test their biologic potential. Cultures exhibiting focal epithelial hyperplasia at the time of implantation continued to exhibit this feature on removal two weeks later. Those retrieved later than two weeks after implantation had reverted to normal histology. The ability of croton oil to promote malignant progression of epithelium in implanted carcinogen-treated cultures is being tested, and immunologically incompetent hosts are being substituted to determine if transformed cells are selectively eliminated.

4) The ultrastructure of carcinogen-treated cultures is being studied to identify markers of malignant transformation before diagnostic histologic changes appear. Lanthanum staining and freeze fracture reveals extensive atypical junctions in the epithelium. Membrane associated alkaline phosphatase and other surface characteristics are being studied. The appearance of abnormal structural proteins and enzymes and deviations from normal cell cycle kinetics are also being assessed. Comparisons are made with untreated cultures, normal tracheal epithelium, regenerating tracheal epithelium, tracheas treated in vivo with benzo(a)pyrene, and tumors arising in tracheas in vivo.

5) Cells are grown in monolayer cultures derived from carcinogen-treated cultures. The growth characteristics and ultrastructure of these cells are compared with those from normal freshly excised tissues, tumors, and from untreated cultures. After carcinogen exposure, explantation is more vigorous, but no gross alteration in cytology is observed. Methods for studying cell surface and cytoskeleton of the populations have been devised and comparisons are under way. Enlarged populations derived by monolayer culture of cells from carcinogen-treated organ cultures will be assessed for tumorigenicity.

Significance to Biomedical Research and the Program of the Institute: The tracheobronchial epithelium gives rise to more fatal human tumors than any other tissue. Yet experimental access to many parameters of carcinogenesis under controlled conditions, including dependable tests of malignant transformation, has been limited. Long-term organ culture of tracheobronchial epithelium constitutes a model which preserves the biological organization of the tissue while permitting control of almost all variables affecting population kinetics and cellular differentiation, stages in carcinogenesis and modification of carcinogen effects by other agents. This contract is directly concerned with the problems of determination of causes of lung cancer, with the early diagnosis of lung cancer lesions, and with effective prevention of lung cancer.

Proposed Course: The proposed course is to transplant rings which have been transformed during culture in media containing BP, to monitor the hosts for metastasis, and to examine recovered transplanted rings and the surrounding tissue for invasive growth. At the same time, methods for monitoring early transformation in vitro including ultrastructural changes, binding of carcinogen in the target cell, karyotype differences, changes in the phases and timing of cell cycles, appearance of new antigens, deletional mutations observed by histochemical, biochemical or immunological techniques, and changes in sensitivity to mitogens are to be studied. A technique for culturing the populatic of epithelial cells which Contractor can now isolate will be sought in order to facilitate some of these studies, especially those involving biochemical changes. The ability to stimulate proliferation of carcinogen-induced cell populations with promoters and to inhibit their growth with vitamin A will be studied.

Date Contract Initiated: June 25, 1973

Current Annual Level: $129,385

STOCKHOLM, UNIVERSITY OF (NO1-CP-33363)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lars Ernster

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this contract is to define the relationship between metabolism of polycyclic hydrocarbons and pulmonary neoplasia. Specific aims are 1) the determination of the cellular distribution of aryl hydrocarbon monooxygenase and of its substrates and products in pulmonary tissue; 2) subcellular fractionation of lung cells and subcellular localization of aryl hydrocarbon monooxygenase activity; 3) development and employment of a highly sensitive assay for aryl hydrocarbon monooxygenase, including studies of tissue disruption to optimize enzymatic activity accessible to assay; 4) dissociation of the cells of the lung and isolation of individual cell types; 5) characterization of the lung aryl hydrocarbon monooxygenase system, including induction of this system, identification of the metabolites of polycyclic hydrocarbons in pulmonary tissue, and charcterization of the binding of these metabolites to DNA; 6) isolation and characterization of lung cytochrome p-450; 7) improvement of the assay for pulmonary epoxide hydrase activity and characterization of this enzyme in much the same manner as aryl hydrocarbon monooxygenase was characterized; 8) improvement of the assay for pulmonary glutathione S-epoxide transferase and isolation and characterization of this enzyme; 9) studies on polycyclic hydrocarbon metabolism in isolated intact hepatocytes and lung cells; 10) studies on the manner in which reactive metabolites of polycyclic hydrocarbons travel from the site of generation to the "target site"; and 11) initial studies on the metabolism of polycyclic hydrocarbons in human lung and skin.

Major Findings: 1) A sensitive and convenient radioactive assay for benzpyrene monooxygenase has been developed based on the use of tritium-labeled substrate and separation of substrate from products by a simple extraction procedure. 2) The applicability of a direct fluorometric assay for benzpyrene monooxygenase to rat lung tissue has been investigated. 3) The new radioactive assay has been employed in studies of various aspects (including kinetics, inhibition, effect of inducers, nature of the products) of benzpyrene metabolism by isolated intact rat hepatocytes. 4) The fluormetric assay has been used to investigate the kinetics and inducibility of benzpyrene monooxygenase in rat lung. 5) A method has been developed for removing hemoglobin from lung microsomes by chromatography

on Sepharose, and another method has been developed to allow the measurement of cytochrome P-450 in the presence of hemoglobin. 6) The spectral properties of cytochrome P-450 have been investigated in lung microsomes from which hemoglobin has been removed. 7) Lung cytochrome P-450 has also been isolated and the properties of the purified form studied. 8) Subfractionation studies on lung microsomes have been carried out involving selective aggregation of fragments of the endoplasmic reticulum with cesium ions. 9) A sensitive modified assay for epoxide hydrase has been developed and used to characterize this activity in lung tissue. 10) Published assay procedures for glutathione S-epoxide transferase have been made more sensitive and isolation of this enzyme from the lung has been started. 11) Levels of glutathione and of various glutathione-metabolizing enzymes in the lung have been measured and compared to the corresponding levels in liver. 12) Lung microsomes have been shown to metabolize 3-hydroxybenzpyrene to a product that binds covalently to DNA. 13) Liver nuclei have been shown to metabolize benzpyrene to products which bind covalently to DNA. 14) The possibility that benzpyrene monooxygenase and epoxide hydrase form a complex in the membrane of the endoplasmic reticulum has been investigated by subfractionation of microsomes. 15) The nature of the benzpyrene metabolites which bind to DNA in isolated, intact hepatocytes has been investigated spectrofluorimetrically. 16) The products of benzpyrene metabolism by total, rough, and smooth microsomes from the liver and lung of rats before and after methylcholanthrene induction have been characterized using high pressure liquid chromatography. 17) The nature and relative importance of conjugation in benzpyrene metabolism by isolated, intact hepatocytes has been studied.

Significance to Biomedical Research and the Program of the Institute: Many of the foreign compounds which enter the human body do so through the lungs. Among these are the polycyclic hydrocarbons, which may be major causes of some human cancers. The principal pathway for metabolism of the polycyclic hdyrocarbons in mammalian tissues involves the inducible enzyme system aryl hydrocarbon monooxygenase, epoxide hydrase, and glutathione S-epoxide transferase. This contract and others in the program are directed towards a detailed understanding of the nature and function of these enzymes and their relationship to carcinogenesis in the respiratory tract.

Proposed Course: 1) To isolate different cell types from rat lung in order to ascertain the cellular distribution of the different enzymes of polycyclic hydrocarbon metabolism and to study hydrocarbon metabolism in these cells; 2) to develop requisite methodology in liver systems for application to lung; 3) to isolate and characterize glutathione S-epoxide transferase from rat lung; 4) to study the ability of lung cell nuclei to metabolize polycyclic hydrocarbons to products capable of binding covalently to DNA; 5) to use reconstituted systems to investigate the relative importance of the three major ways in which arene oxides are transformed into less carcinogenic products (i.e., hydration by epoxide hydrase, enzymatic and non-enzymatic conjugation with glutathione, and spontaneous isomerization in water to phenols).

Date Contract Initiated: June 25, 1973

Current Annual Level: $185,000

TENNESSEE, UNIVERSITY OF (NO1-CP-33369)

Title: Maintenance of Organ Explants from Rodent Pancreas

Contractor's Project Director: Dr. Leonard R. Murrell

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: Organ culture is increasingly being utilized as a means of bridging the gap between cell culture and in vivo conditions. Differentiated tissues maintained in organ culture permit direct histological and/or biochemical analysis of the influence of experimentally administered compounds (including carcinogens) on the tissue itself, and simultaneously permit examination of products released by the cultured tissue into medium used for culture.

Postnatal acinar pancreas, unlike many organs, has been difficult to maintain in organ culture. The present contract has been devoted to devising appropriate methods for preserving functional and structural integrity of mature pancreatic tissue during organ culture periods ranging from several days to several weeks. Contractor's approach has been to examine the effects of a number of system dependent physical and chemical culture variables under well-controlled experimental conditions in order to approach a definition of the "optimal" culture conditions for this organ.

Major Findings: Culture methods developed during early phases of this contract involved a circumfusion apparatus by means of which culture medium could be exchanged at frequent intervals or a rocker culture method with less frequent medium change. Either approach minimizes tissue changes resulting from toxic microenvironments created by release of proteolytic enzymes or other tissue products during culture. By using a chemically defined (serum free) culture medium, explants prepared from 7 to 15 day postnatal, rat pancreas can now be maintained for periods of at least 3 weeks with retention of pancreatic specific function (monitored by amylase content and release) and characteristic morphology. Among the variables optimized earlier were gas phase oxygen, medium pH, medium exchange rate, and explant size. In addition to these variables, it has been demonstrated that long-term preservation of this organ is superior when the medium contains glucocorticoid, ascorbic acid, and $FeSO_4$ in addition to the components normally in Trowell's T8 medium. Addition of HEPES buffer assists in maintenance of appropriate pH.

Cultures under the conditions outlined appear to remain functional (estimated by increased amylase output in dose-dependent response to pancreozymin), and cells within the cultures synthesize DNA (autoradiography following

pulse tritiated thymidine labeling). During the longer culture periods, a gradual loss of mature pancreatic acini is observed with an apparent concomitant increase in pancreatic duct-like elements. Pancreatic islets remain throughout the culture period, and (based on insulin assays of medium, and absence of a requirement for exogenously-added insulin or glucagon) are apparently functional.

Potential longevity of cultures under these conditions has not been determined, since few have been examined beyond 21 days of culture. Similarly, the suitability of cultures for studies of chemical carcinogenesis has not been evaluated, since NCI and the Contractor agree that carcinogen treatment is inappropriate under terms of this contract.

Significance to Biomedical Research and the Program of the Institute: This project is one of several initiated in an attempt to develop culture models which might facilitate certain studies of pancreatic carcinogenesis of types currently impractical in intact animals.

Proposed Course: The essential goal of this project—the development of culture methods and media by which pancreatic tissue can be maintained for several weeks in culture—has been accomplished. The contract was terminated October 31, 1976.

Date Contract Initiated: June 29, 1973

Current Annual Level: 0

TEXAS, UNIVERSITY OF (Southwestern Medical School at Dallas) (NO1-CP-33362)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Directors: Dr. Russell A. Prough
Dr. Ronald W. Estabrook

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this contract is to provide basic biochemical studies which may define the relation between the enzyme system, arylhydrocarbon hydroxylase (AHH), and pulmonary neoplasia. Specific aims are: 1) physicochemical and enzymatic characterization of the isolated lung cell fractions of humans and rodents, 2) analysis of lung metabolism of benzo(a) pyrene and its metabolites using high pressure liquid chromatography, and 3) analysis of benzo(a)pyrene metabolism by isolated cells from rodent lung.

Major Findings: The research approach taken in this study has been to establish techniques to analyze the total metabolism of benzo(a)pyrene using sophisticated instrumentation, including high pressure liquid chromatography, turbid sample visible spectrophotometry, and electron spin resonance spectrophotometry. The formation of benzo(a)pyrene phenols has been directly observed using turbid sample visible spectroscopy and is inhibited by

typical monooxygenase inhibitors, such as, 7,8-benzoflavone and metyrapone. The analysis of total benzo(a)pyrene metabolites from incubation mixtures containing liver and lung microsomes from rodents and humans has been performed,and these studies support the previous reports of this contract that the lung AHH activity is a minor monooxygenase activity of lung microsomal fractions from rodents and humans. Experiments related to the time course of metabolism by lung microsomes suggest that differences do exist between liver and lung other than the rates of formation. Currently, the metabolism of the primary oxidation products of benzo(a)pyrene is being studied to evaluate any substrate specificity differences between the two organs. Electron spin resonance studies coupled with gas chromatography-mass spectrometry and nuclear magnetic resonance spectrometry support the suggestion that certain benzo(a)pyrene phenols can be oxidized, either chemically or enzymatically, to yield products with free radical signals. The further metabolism of benzo(a)pyrene to reactive intermediates is being studied to assess both the extent of metabolism and the reactivity of secondary metabolites.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons and their metabolites are recognized as potential carcinogenic agents. A major route of introduction of polycyclic hydrocarbons into the human is through the respiratory tract. The primary mechanism of metabolism of polycyclic hydrocarbons involves oxygen activation by a unique hemoprotein-containing electron transport system associated with the endoplasmic reticulum of many tissues. This contract and others in the program are directed toward a detailed understanding of this enzymatic system functional in the oxidative metabolism of polycyclic hydrocarbons and its role in respiratory tract carcinogenesis.

Proposed Course: The metabolism studies on benzo(a)pyrene and its primary metabolites in human and rodent lung will be continued using high pressure liquid chromatography techniques capable of monitoring total metabolism; in addition, the binding of reactive intermediates to DNA or other nucleophiles will be monitored. These studies will subsequently be expanded to include analysis of benzo(a)pyrene metabolism by isolated cells from human and rodent lung.

Date Contract Initiated: June 20, 1973

Current Annual Level: $51,700

TORONTO, UNIVERSITY OF (NO1-CP-75879)

Title: Biochemical and Morphologic Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. E. Farber

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective is to explore further the essential nature and properties of the early putative preneoplastic cell populations

induced in the liver by chemical carcinogens with a view to the development of rapid in vivo bioassay procedures for chemical carcinogens and procedures for the identification of early preneoplastic cells in the intact animals. Included in the objectives are: a) purification, characterization, and assay of a liver preneoplastic antigen, PN-antigen, that might prove useful in the quantitative assay of preneoplastic liver cells and in the further in-depth analysis of the nature of the preneoplastic changes; and b) the in-depth analysis of initiation and promotion in the liver with biological and biochemical approaches as a base for the development of a valid reliable rapid bioassay for carcinogens in intact animals.

Major Findings: As this contract was only recently initiated, there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute: The proposed research has the real possiblity of developing a new approach to the rapid bioassay of chemical carcinogens in vivo, based on initiation of potential cancer cells. This, coupled with a rapid in vitro assay such as the Ames test, would allow for a more acceptable rapid screen for carcinogens since it includes an animal assay.

In addition, the research has the possibility of developing a test for early preneoplastic cells, based on the appearance of placental hormones and enzymes in such cells. The conditions for the release of these from the cells are now being sought to enable this to be pursued.

Proposed Course: Contractor plans to pursue the purification of PN antigen, followed by the development of a radioimmunoassay. Contractor also plans to observe whether human chorionic gonadotropin (B-subunits) (HCG) and placental lactogenic hormone can be used for the quantitative assay of preneoplastic and neoplastic cells in liver and whether, through the use of butyrate or other compounds, these new constituents can be released into the blood. If this can be achieved, a new approach to the very early diagnosis of preneoplasia becomes possible.

Date Contract Initiated: February 1, 1977

Current Annual Level: $83,306

UTAH, UNIVERSITY OF (NO1-CP-55709)

Title: Quantitation of Physiologic Reflux in Pancreatic Duct of Primates

Contractor's Project Director: Dr. Frank G. Moody

Project Officer (NCI): Dr. Carl E. Smith

Objectives: These studies are designed to identify conditions which encourage reflux of bile from the bile duct into the duct of Wirsung (pancreatic duct)

of unanesthetized Rhesus monkeys. This contract period has focused on quantitating the frequency and amount of retrograde movement of a radioactive marker from the gallbladder through the bile and pancreatic ducts into a jejunal pouch attached to the tail of the pancreas. A model for assessing biliary pancreatic reflux had been developed previously.

Major Findings: Hypaque (a radiopaque solution) instilled in the gallbladder entered the pancreatic duct in 19 of 30 studies (15 monkeys). Radiographic reflux occurred in six studies in which the jejunal fistula pressure was raised to 30mm Hg, a pressure in excess of that measured in the common bile duct. Radioactive polyethylene glycol (^{14}C-25 μCi/ml) was placed in the gallbladder of four monkeys during 26 studies. When compared to a control group (six studies, four monkeys), there was a significant rise in fistula counts beginning 60 min after instillation into the gallbladder. Counts rose from 2.4 ± 0.2 CPM (n=52, SEM) to 15.7 ± 1.6 CPM (n=52, $p<.001$). Fistula radioactivity returned to baseline at six hours. The rise and fall of radioactivity inscribed a uniform, single peak frequency distribution curve. Contractor interprets these data to mean that the movement of bile through the pancreatic duct is a simple, quantifiable biologic function.

Significance to Biomedical Research and the Program of the Institute: Work thus far provides the biomedical community with a primate model for studying the conditions which enhance flow of bile from the biliary tree into the pancreatic ductal system. The time-flow characteristics of bile reflux have been quantitated under relatively physiologic conditions. These studies suggest that biliary pancreatic reflux may be a relatively common event in monkeys. If this is also true in humans, then constituents of bile in contact with pancreatic ductal epithelium must be suspect as potential inducers of pancreatic cancer.

Proposed Course: It now remains to identify conditions which enhance or prevent biliary pancreatic reflux. Utilizing the pancreatic-jejunal fistula model, and the polyethylene glycol reflux techniques described above, monkeys will be administered graded doses of secretin, gastrin, cholecystokinin, and glucagon in an attempt to characterize hormonal effects on biliary pancreatic reflux. Meals of varying composition will also be studied. A major goal of the coming year is to accurately quantitate the ratio of duodenal loss to pancreatic reflux under the conditions described above.

Date Contract Initiated: June 29, 1975

Current Annual Level: $107,603

VERMONT, UNIVERSITY OF (NO1-CP-33360)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. John E. Craighead

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: This project has five basic objectives: 1) the development of organ culture systems which permit viability and differentiation of hamster tracheal epithelium for extended periods of time; 2) the development of techniques for the application of carcinogens quantitatively to respiratory tract epithelium in organ culture; 3) the development of techniques for the transplantation of carcinogen-treated organ culture tissue into susceptible hosts to document in vivo neoplastic transformation due to carcinogen exposure in vitro; 4) the monitoring of animals with implants of carcinogen-treated organ cultures for the appearance and progression of epithelial enoplasms; and 5) the histologic and ultrastructural characterization of neoplasms that develop in animals.

Major Findings: The experimental systems and approaches developed in previous years of contract support were used to establish reproducible organ culture systems for the evaluation of carcinogens and cocarcinogens. Significant accomplishments during the year were as follows: 1) Studies of the nutritional requirements of respiratory tract tissue were completed. Recent experiments show that nutritional influences (nucleic acid precursors, amino acids, vitamins, inorganic salts, insulin, and glutathione) play a role in the development of squamous metaplasia. In a vitamin A-deficient medium, non-essential amino acids promote and strikingly enhance the development of squamous metaplasia. The amino acids serine and glutamic acid have more pronounced effects than other non-essential amino acids.

2) An understanding of the interaction of environmental dusts (crocidolite and amosite asbestos, carbon, ferric oxide) with differentiated cells of the tracheal mucosa was advanced. In organ culture experiments, effects of particles of defined size and composition were evaluated over a range of concentrations. Particulates of several types promoted epithelial hyperplasia. Interestingly enough, the response of the respiratory epithelium to each of these dusts has unique features. At high concentrations, the materials were cytotoxic, whereas at lower concentrations, a variety of cytopathic changes were observed.

3) Anaplastic carcinomas were induced regularly in hamsters implanted with organ cultures that had been exposed to inorganic dusts and 3-methylcholanthrene (3 MC). Initial studies using ^{14}C-3 MC showed that the hydrocarbon could be bound quantitatively at low temperatures to the particles. This made it possible to quantitate both the dust and carcinogen exposure to the organ culture and determine the release of hydrocarbon after intervals in culture. Thus far, the cocarcinogenic effects of ferric oxide and crocidolite asbestos have been compared. With asbestos, as opposed to ferric oxide, it is possible to induce tumors with 10 to 20-fold lower concentrations of 3 MC (i.e., < .1 μg 3 MC/4mm^2 epithelial surface).

4) Addition of vitamin A analogs (retinoids) to cultures of tracheal explants exposed to amosite or crocidolite asbestos showed that retinoids inhibit

augmented DNA synthesis in the tracheal epithelium. In Contractor's initial studies, the effects of 13-cis retinoic acid (Hoffman-LaRoche) on organ cultures were explored after exposure to amosite. However, the retinoid was toxic to the tracheal epithelium at both the concentrations employed (ca. 10^{-6} and 10^{-7} M). In subsequent experiments, the retinoid retinyl methyl ether was added at a lower range of dosages to cultures after exposure to either amosite or crocidolite asbestos. This compound inhibited incorporation of ^{3}H-thymidine without inducing degenerative changes in the mucosa.

5) Synthetic fibers were used to evaluate carcinogen dosage to the epithelium over extended periods of time. Tracheal hemisections were maintained in organ culture, an ^{14}C-3 MC-coated Lycra fibers were applied to the epithelial surface. These cultures were subsequently implanted into hamsters after removal of the fiber. In parallel, fibers coated with comparable amounts of 3 MC were inserted into excised, intact hamster tracheas which were than grafted on syngeneic animals (Kendrick et al., J. Natl. Cancer Inst. 52: 1317, 1974). The organ cultures, in general, failed to develop histopathologic alterations indicative of preneoplastic or neoplastic change. Carcinomas appear regularly in tracheal grafts. This evaluation suggests that a continuous carcinogenic stimulus must be applied to the respiratory epithelium in vivo in order to induce malignant transformation.

6) Organ cultures were found to be an effective means for evaluating alterations in the normal morphology and replicative rate of the tracheal epithelium after addition of water soluble carcinogens (NMU, MNNG, and DMN). Toxicity of the three test compounds was variable but dose-dependent.

Significance to Biomedical Research and the Program of the Institute: A variety of physical and chemical substances, including asbestos, ferric oxide,and polycyclic hydrocarbons have been identified as respiratory tract carcinogens or cocarcinogens after their administration to laboratory animals. As it is unclear how these agents or combinations of agents affect the tracheobronchial mucosa, detailed studies of carcinogenesis at the cellular level are needed. Organ culture of respiratory tract tissue affords a means for studying carcinogen and foreign body interaction with the differentiated epithelial cell.

Proposed Course: To conduct further studies in which quantitatively determined amounts of carcinogens are bound to particulates (carbon, asbestos, ferric oxide) or synthetic fibers. Specific goals are: 1) to elucidate further the mechanisms of carcinogenesis and conduct a comparative assessment of preneoplastic and neoplastic changes in carcinogen-treated explants maintained in vivo and in vitro; 2) to define carcinogen-cocarcinogen (i.e., particulate) dosage relationships in respiratory tract tissue and how particulates play an adjuvant role in tumor development in the respiratory epithelium; 3) to develop additional model systems for the induction of carcinomas in organ cultures and tracheal grafts,

using tissue from selected rodent species; and 4) to determine whether or not continuous carcinogenic and cocarcinogenic stimulation in vivo and in vitro is required for transformation of differentiated respiratory epithelial cells.

Date Contract Initiated: June 28, 1973

Current Annual Level: $110,427

VETERANS ADMINISTRATION HOSPITAL (Tampa, Florida) (YO1-CP-55625)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor's Project Directors: Dr. Hollis G. Boren
Dr. Louis J. Paradise

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: To determine the proliferative cellular response of segments of the respiratory tract following exposure to respiratory carcinogens and particulates using primarily the hamster intratracheal instillation model. These measurements are correlated with the histopathological response to carcinogens and particulates. Radioactive carcinogens, anti-carcinogens, and other compounds are localized in the respiratory tract, and their amounts are determined by quantitative light microscopic auto-radiography. Serial sacrifice studies are performed to define the morpho-genesis of squamous cell carcinoma of the lung, as well as the determination of which preneoplastic lesions are modified by vitamin A compounds.

Major Findings: Cell kinetics of tracheal epithelial cells in normal, adult male hamsters have been measured by four methods. The fraction of mitoses labeled with ^{3}H-thymidine and the double labeling method in which ^{3}H-TdR is followed by ^{14}C-TdR provided complementary results for durations of DNA synthesis (S) and post-synthetic gap (G2). From double labeling experiments, duration of mitosis (M) was determined. In order to measure cycle time and growth fractions, two different methods were required: continuous labeling with ^{3}H-TdR and grain counts over inter-phase nuclei at time intervals to 17 days following a single ^{3}H-TdR injection. To identify basal, mucous, and ciliated cells precisely, 1μm sections of trachea were used for emulsion autoradiography. Effects of cell synchrony were avoided by maintaining hamsters under conditions of constant room light.

For fraction of labeled mitoses, times of sacrifice were from 1 to 36 hours after pulse labeling (intraperitoneal). For double labeling, 17 different time intervals between injections were used. Both basal and mucous cells incorporated thymidine and were observed in mitosis, but ciliated cells were not labeled with these two methods nor were they observed in mitosis. Continuous labeling at 6-hour intervals for 8 days

with groups of hamsters sacrificed every 2 days demonstrated that 20 percent of basal cells were labeled at each sacrifice period. Numbers of mucous cells labeled were much lower and increased gradually with increasing time. Regression of number of labeled mucous cells on time allowed calculation of growth fraction (Y-intercept) and cycle time (X-intercept). Quantitative autoradiographic methods which consist of standardization of grain counts from one slide to another by means of grain counts over 1μm sections of ^{3}H-poly(n-butyl)methacrylate were used for the fourth method. Stepwise grain-halving was not observed. Instead, there was a linear decrease in mean grain counts over both basal and mucous cells with increasing time after injection of ^{3}H-TdR in semilogarithmic plots. The method used for calculating cycle time takes advantage of the changes in coefficients of variation of the grain counts with increasing time. From mean grain counts, at times shortly after the first cell division following ^{3}H-TdR and at times to 17 days after ^{3}H-TdR, the fractions of labeled cells which have not divided again, have divided once, and have divided twice are determined. Cycle time is calculated from this last fraction.

Definitive values have now been established. Cell cycle values for basal cells were S = 10.8 ± 1.5 hr; G2 = 3.2 hr; M = 0.6 ± 0.1 hr; G1 = 109 hr; cycle time = 124 ± 20 hr; growth fraction = 0.2; labeling index = 0.011; mitotic index = 0.00043 ± 0.00004. Values for mucous cells were S = 7.0 ± 2.0 hr; G2 - 2.5 hr; M = 0.6 ± 0.15 hr; G1 = 93 hr; cycle time = 103 ± 14 hr; growth fraction = 0.03; labeling index = 0.004; mitotic index = 0.00024 ± 0.00003. From grain counts over interphase cells at 3 months after administration of ^{3}H-TdR, the fraction of cells dividing very slowly or "resting" (G0) was calculated as 0.0002 for basal cells and 0.0003 for mucous cells. From mean grain counts and labeling index at 3 months after ^{3}H-TdR, lifespans of cells were estimated.

Labeled ciliated cells were present by 10 days after pulse labeling with ^{3}H-TdR. Normalized mean grain counts for ciliated cells were not significantly different from those of mucous cells at 10, 14, and 17 days after ^{3}H-TdR, but were significantly higher than those of basal cells at these times. Frequency distributions of normalized grain counts for ciliated cells resembled distributions for mucous cells more closely than distributions for basal cells. Distributions for ciliated cells were significantly different from those of basal cells at 14 and 17 days, but were not different from mucous cells. These findings suggest that ciliated cells arise by differentiation of mucous cells rather than of basal cells.

From labeling indices at times from 10 days to 84 days after ^{3}H-TdR, lifespans of cells were estimated. For basal cells, lifespan was 104 days; for mucous cells, it was 119 days; for ciliated cells, it was about 130 days.

Significance to Biomedical Research and the Program of the Institute:
This contract is an integral part of an interlocking group of contracts designed to define the roles of carcinogens and the physicochemical factors (e.g., particulates) required for respiratory carcinogenesis. The other

tudies, including long-term tests, have been designed so that they are closely related to the present one with maximum interaction, but no overlap of activity, in an effort to define the key roles of vehicles and particulates in the process of respiratory carcinogenesis. The cell kinetics studies for tracheal epithelium provide a basis for measurement of biological effects of carcinogens and conditions inhibiting carcinogenesis.

Proposed Course: A combination of continuous labeling, double labeling, and normalized grain count methods is being used to study cell kinetics of tracheal epithelium exposed to N-nitroso-N-methylurea in limited areas. Alterations in cell kinetics of tracheal epithelium in exposed areas will be determined and will be correlated with histologic changes and with the time of appearance of tumors.

Date Contract Initiated: June 2, 1972

Current Annual Level: $132,910

VETERANS ADMINISTRATION HOSPITAL (Washington, D.C.) (YO1-CP-60204)

Title: Studies on Normal, Premalignant, and Malignant Respiratory Epithelium of Humans

Contractor's Project Director: Dr. Paul W. Schafer

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: 1) Acquire and provide to Program normal, preneoplastic, and malignant human surgical specimens (primarily bronchial tissues) for in vitro studies; and 2) perform morphological studies of these human tissues at the light and electron microscopical levels.

Major Findings: Contractor has supplied to the Program bronchial and parenchymal tissues obtained from twenty-two patients undergoing lung resection for tumors and other diseases and has initiated morphological studies as planned.

Significance to Biomedical Research and the Program of the Insitute: The nature of premalignant changes in human tissues is poorly understood. Tissues provided from this contract will be used in a number of studies in vitro to evaluate the response of human tissues to carcinogens and to evaluate oncogenic potential of treated tissue in xenotransplantation studies in nude mice.

Proposed Course: a) To continue acquisition and supply of human tissues to Program and b) to continue morphological studies of the human tissues; in particular, to contribute to the establishment of a classification of lung tumors based on ultrastructural microscopy and other studies in addition to paraffin ligh microscopy.

Date Contract Initiated: March 1, 1976

Current Annual Level: $29,138

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NO1-CP-55685)

Title: Relationship of Fecal Neutral Sterols to Large Bowel Cancer Risk

Contractor's Project Director: Dr. Tracy D. Wilkins

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To study factors which affect bacterial metabolism of neutral steroids in the human colon, including the physicochemical state of the steroids and the presence of dietary fiber and mucin.

Major Findings: Concentrations of long chain fatty acids, compounds which could affect the solubility of neutral steroids in the colon, were determined in the feces of two high risk (North Americans and polyp patients) and two low risk populations (rural Japanese and Africans). Significantly higher concentrations of oleic acid (C18:1) were excreted by the two high risk populations. Reduction of cholesterol by an organism isolated previously from human feces was inhibited by the presence of strains of Bacteroides and a Fusobacterium species. Since the availability of carbon sources may influence cholesterol reduction, strains of Bacteroides and other major genera from the colon were surveyed, and it was found that a number of species ferment plant polysaccharides (dietary fiber components) and mucins which are present in the colon. Polysaccharide degrading enzymes produced by Bacteroides were demonstrated to be cell-associated and inducible. Analysis of colon contents has confirmed the fact that mucin and dietary fiber are degraded in the colon, and the products of bacterial degradation of these substances may have altered capacity for adsorption of neutral steroids and bile acids. Desulfomonas pigra, an intestinal bacterium which uses sulfate rather than cholesterol as a terminal electron acceptor, was shown to have cytochromes.

Significance to Biomedical Research and the Program of the Institute: Determination of the way in which components of the colon contents (lipid, carbohydrate) interact with bacterial metabolism of neutral steroids is necessary for an understanding of the role of neutral steroid metabolism in vivo and of the fate of the steroid metabolites in the colon.

Proposed Course: The relationship of bacterial degradation of fiber and mucin to the bacterial alteration of neutral steroids will be investigated further. Mutagenicity and steroid metabolites will also be studied.

Date Contract Initiated: June 1, 1975

Current Annual Level: $129,839

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NO1-CP-33334)

Title: Comparative Fecal Flora Studies

Contractor's Project Directors: Dr. W. E. C. Moore
Dr. L. V. Holdeman

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To determine the microbiological differences in the fecal and intestinal flora of human populations with high risk of colon cancer as compared with the flora of persons in populations with low risk of colon cancer.

Major Findings: The composition of the fecal flora of populations at high risk and at low risk of colon cancer has been determined. The high risk populations sampled include: a) patients from whom adenomatous polyps have been removed, b) a cohort group of Japanese-Hawaiians, and c) (in a related project) "normal" persons from the continental U.S.; the low risk populations sampled include native rural Africans and native rural Japanese. Cultural characteristics of 6271 microbial strains isolated from these populations are now accessible from a computer file for analysis of the characteristics of strains isolated from the different populations, as well as for analysis of species isolated from the various populations.

Contractor has found no significant difference in the ratio of bacteroides and bifidobacteria among the two risk groups. Although the North Americans and polyp patients have more bacteroides and bifidobacteria than do the low risk Africans, the other low risk group (rural Japanese) has more bacteroides and bifidobacteria than do the North Americans and polyp patients. There are, however, differences in the species that are predominant in the two risk groups. Correlated with increased risk are Bacteroides vulgatus, Bacteroids distasonis, and Peptostreptococcus productus. Correlated with low risk are Eubacterium aerofaciens II, Bacteroides fragilis, and Escherichia coli. The low risk populations do appear to have more facultative bacteria than do the high risk groups, and the high risk populations have more strains that grow well in laboratory media containing bile than do the low risk group. There was no readily apparent difference between the two groups in the percentage of the isolates that produce indole.

Studies of recovery from duplicate cultures shipped as air cargo and carried in the passenger cabin from South Africa indicated that the shipping of cultures did not adversely affect the results if one discarded from consideration isolates from shipped cultures that were obviously spreading contaminants of other colonies.

Other findings resulting from Contractor's studies of human intestinal flora indicate: a) each individual tends to maintain his own fecal flora, which is different from that of another individual in the same population; b) the diet of the host does not exert a major influence on the composition of the flora; and c) no anatomical area of the normal gastrointestinal tract

is sterile, and major bacterial growth occurs in the cecum and ascending colon, after which the total counts and types of organisms present in the lower colon (and feces) remain constant.

Significance to Biomedical Research and the Program of the Institute: Documentation of the differences in flora and the physiological properites of the organisms should indicate the probable role of these organisms and their metabolic products (perhaps in response to certain substrates) in colon cancer. In addition to colon cancer, results from this research may also relate to atherosclerosis, obesity, and nutrition.

Proposed Course: Analysis of the data of the flora of the different risk groups is continuing. Differences in metabolic activities, as well as in species present, are being investigated. Complete statistical analyses must be done to determine the significance of the observations reported above.

Date Contract Initiated: June 1, 1973

Current Annual Level: $137,942

W. ALTON JONES CELL SCIENCE CENTER (NO1-CP-65762)

Title: In Vitro Cultivation of Normal, Prostatic Epithelial Cells

Contractor's Project Director: Dr. William H. J. Douglas

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: a) To establish primary cultures from the ventral lobe of rat prostate and b) to characterize these primary cultures to determine the specific properties of normal, prostatic epithelium that are retained in vitro.

Major Findings: The majority of the efforts have been directed toward techniques to isolate prostatic epithelial cells from rat ventral prostate and toward identification of markers specific for prostatic epithelium.

Several enzymes were tested for digestion of the prostate: collagenase, dispase, thermolysin, and a collagenase-trypsin-chick serum mixture (CTC). The efficacy of each digestion method was assayed by 1) number of viable cells/gram of tissue, 2) growth when seeded at 10^5 cells/plate, and 3) growth when seeded at 10^3 cells/plate. At 10, 30, 50, and 70-minute intervals the cells were harvested and the viable cell count determined by trypan blue exclusion. Viable cells were seeded into 60 mm dishes containing F12K medium with 10% fetal calf serum (FCS) at 10^3 or 10^5/plate. CTC gave the most complete digestion with the highest cell yield. However, cells dissociated with CTC had the lowest attachment rate with about 20-50 colonies/plate seeded at 10^3 cells/plate. Collagenase and dispase yielded cells with greater attachment efficiency, about 250 colonies per plate. All enzymes yielded cells with various morphologies. A few colonies of solely epithelial-like cells were noted from a CTC digestion.

Several growth media were evaluated for cells dispersed by the CTC enzyme mixture: F12K, MCDB 201, and MEM with non-essential amino acids and CMRL 1066. All media were supplemented with 10% FCS (GIBCO) and contained penicillin (100 unit/ml), streptomycin (100 µg/ml), and amphotericin B (2 µg/ml). F12K and MEM supported growth of the largest number of clones per plate and maximal growth in mass culture.

When monodisperse prostate cells were seeded into culture vessels at 10^5 cells/plate many of the cells did not attach to the culture surface during the first 24 hours. The medium was removed from the mass culture plates after 24 hrs. incubation and transferred to new dishes. Within 24-48 hrs. after transfer to a new culture dish, the floating cells attached to the culture surface and established small patches of epithelial-like cells among many fibroblast-like cells.

Attempts were made to encapsulate prostatic tissue with epithelial cells. Several pieces of the tissue were then placed in plastic dishes and allowed to attach. After one day of incubation, epithelial-like cells had migrated from some of the tissue pieces, as determined by light microscopy.

Procedures have been developed for histochemical localization of acid phosphatase activity and zinc in frozen sections of rat ventral prostate and in cell cultures derived from monodisperse suspensions of ventral prostate.

Significance to Biomedical Research and the Program of the Institute: One of the major goals of the National Cancer Institute is to determine the early changes that occur in prostatic epithelium prior to the onset of adenocarcinoma. This is impossible to do in patients. However, the availability of diploid primary cell cultures from normal human and animal prostatic epithelial cells will permit study of the initial stages during induction of adenocarcinoma. In addition, these cultures will permit one to evaluate the influence of hormones, drugs, and carcinogens on prostatic epithelium.

Proposed Course: Studies during the next year will concentrate on: 1) Markers: identification of specific markers that may be used to identify prostatic epithelial cells in vitro. 2) Growth Promotion: development of cell culture media which specifically promotes the growth of diploid prostatic epithelial cells in vitro.

Date Contract Initiated: September 30, 1976

Current Annual Level: $107,164

WEST VIRGINIA UNIVERSITY (NO1-CP-75887)

Title: Prostate Transplant Model: A System for Studying Neoplasia

Contractor's Project Director: Dr. John A. Thomas

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To study the effect of age and hormone environment on the prostate cell in order to develop a model for adenocarcinoma of the prostate. Specific objectives include an evaluation of the changing endocrinology of the aging rat ventral prostate transplant and the in situ organ. Efforts will be made to compare nucleic acid synthesis in transplanted tissue to that occurring in situ. Additional efforts will be made to correlate the biochemical and morphologic changes (e.g., epithelial cell height and secretory activity) in the ventral prostate transplant and in the host's in situ organs.

Major Findings: Since the current NCI contract was initiated on February 28, 1977, sufficient time has not yet elapsed for examining 6 and/or 12 weeks post-transplantation studies.

Significance to Biomedical Research and the Program of the Institute: The etiology of adenocarcinoma of the prostate gland remains unknown. Unfortunately, there is no ideal animal model in which to elucidate the underlying biochemical and morphological changes involved in this pathological condition. These studies will be directed toward not only producing an abnormal prostate in animals but also to trying to learn how the aging cell in this gland becomes neoplastic.

Proposed Course: Recently completed studies have determined some of the significance of host age, donor age, and the duration of transplantation on prostate transplant survival.

A primary objective will be a histological evaluation of the hormonal status of the aging rat and to what extent it influences ventral prostate gland transplants and the same in situ lobe of this gland in the host. To achieve this objective, ventral prostate glands obtained from aged 54 weeks old Fischer (F344) male rats will be transplanted into the cleared 5th mammary fat pads of 6 weeks old syngeneic hosts and the transplants maintained for 6 and/or 12 weeks. These hosts will be implanted with testosterone propionate pellets. Thus, the variables of age and androgen that are important in the genesis of human prostate cancer will be simulated in this animal model.

The DNA levels and morphological characteristics of the transplanted prostate tissue will be monitored and correlated in an effort to determine if this experimental design leads to the induction of tumors.

Date Contract Initiated: February 28, 1977

Current Annual Level: $59,000

WEST VIRGINIA UNIVERSITY (NO1-CP-43230)

Title: Relationship of Pituitary Hormones and Androgens on Prostate Gland Metabolism

Contractor's Project Director: Dr. John A. Thomas

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: A principal goal is to learn how pituitary hormones like prolactin contribute to the growth and proliferation of the rodent prostate gland. Experiments will attempt to further elucidate the interaction of prolactin and androgens with regard to producing an acceleration of biochemical activities and an altered prostate gland morphology. Recently, the use of a transplant biological system has provided a rather unique procedure in which to study both prostatic morphology and biochemistry. By studying these hormonal interactions, with or without the presence of a chemical carcinogen (e.g., ethylnitrosourea (ENU), dimethylbenzanthracene (DMBA), and methylcholanthrene (MCA), it might be possible to consistently produce a tumorous prostate that can serve as an animal model. Such a model might be useful in elucidating some of the underlying biochemical and histological changes in the abnormal prostate.

Major Findings: A single intraprostatic injection of 7,12-dimethylbenz(a) anthracene (DMBA) (2.5 mg/kg), of 3-methylcholanthrene (MCA) (2.5 mg/kg) or of 1-ethyl 1-1-nitrosourea (ENU) (5 mg/kg) into mature rats (Fischer and Wistar strains) led to alterations in the metabolism of testosterone by various lobes (e.g., ventral, lateral, dorsal, and anterior) of this accessory sex organ. In the Wistar strain, dihydrotestosterone (DHT) formation was reduced by these carcinogens at 6 or 12 weeks post-injection. In the Fischer strain, ENU also caused reduction in DHT formation, but DMBA failed to significantly alter the formation of this metabolite from ^{3}H-testosterone. Regardless of the strain or post-injection intervals, few significant changes were noted in ^{3}H-thymidine incorporation into prostate gland DNA. A histological examination of tissues from carcinogen-treated prostate showed that DMBA, MCA, ENU-treated prostatic epithelia were often hyperplastic. DMBA- or MCA-treated prostate cells often become stratified and underwent squamous metaplasia. These studies revealed some degree of correlation between altered androgen metabolism and histological changes in the prostate gland.

Transplantations of ventral prostates to the cleared 5th mammary fat pads of Fischer (F344) rats revealed that the age of the donor prostatic tissue, the age of the host animals, as well as the duration of the post-transplantation interval influenced the survival of the prostate transplant. The results of these studies suggested that the in situ prostate's biochemical and morphological aspects can be compared with a transplanted prostate, and this can be investigated in young (6-12 weeks) and old (54-90 weeks) hosts and donors. Dihydrotestosterone pellet implantations usually resulted in a greater stimulation of growth, DNA, and steroidogenesis than in those animals implanted with testosterone pellets. When the metabolic activity (e.g., DNA and androgen metabolism) in well-vascularized 12-week old transplants and in the in situ prostates was compared, the metabolism in the host prostate was usually 1 to 3 times greater than in the transplant tissue although both sites seemed to retain their hormonal

sensitivities. Daily injections of ovine prolactin to the host rats did not result in any significant alterations in testosterone metabolism but caused changes in tissue weight in both the transplants and in the in situ ventral prostate.

A histological examination of ventral prostate transplants revealed maintenance of the epithelial cell height and secretory activity as the transplant aged from 6 to 12 weeks. This maintenance of acinar epithelial integrity in these transplants appeared to be somewhat correlated to the levels of radiometabolites of testosterone formed. These findings suggest that the prostate transplant model system can be employed in the study of the aging of prostatic tissue.

Significance to Biomedical Research and the Program of the Institute: The etiology of adenocarcinoma of the prostate gland remains unknown. Unfortunately, there is no ideal animal model in which to elucidate the underlying biochemical and morphological changes involved in this pathological condition. These studies will be directed toward not only producing an abnormal prostate in animals, but they will also be directed toward trying to learn how the aging cell in this gland becomes neoplastic. Hoepfully, such studies will be helpful in understanding prostatic cancer in man.

Proposed Course: Recently completed studies have determined some of the significance of host age, donor age, and the duration of transplantation on prostate transplant survival. Studies in this contract have been satisfactorily completed, and the contract has terminated.

Date Contract Initiated: June 28, 1972

Current Annual Level: $7,361

SUMMARY REPORT

CHEMISTRY OPERATIONAL UNIT

July 1, 1976 through September 30, 1977

The Chemistry Operational Unit provides administrative and program management direction for two complementing Collaborative Research Segments, the *Carcinogen Metabolism and Toxicology Segment*, and the *Chemistry and Molecular Carcinogenesis Segment*. The *Carcinogen Metabolism and Toxicology Segment* has as one of its goals the identification of those components of complex systems which function as environmental carcinogens and the development of analytical methodology for their detection and quantitation. Metabolic and toxicologic studies of environmental carcinogens in selected animal systems are conducted to provide additional knowledge on the metabolic pathways involved in the carcinogenic process. Such studies may also furnish an explanation for the varying responses of different animal species and man to chemical carcinogens. The mechanism of chemical carcinogenesis in selected animal systems with defined characteristics is being investigated. The major objective of the *Chemistry and Molecular Carcinogenesis Segment* is to gain an understanding of the molecular process involved in chemical carcinogenesis and an understanding in chemical biological terms of the important events that lead to carcinogenesis.

Studies in the *Metabolism and Toxicology Segment* have been grouped into two major approach areas: (1) the Nitrosamines Program; and (2) Mechanistic and Metabolic Studies of Carcinogens, the major aspects of which are described below.

Nitrosamines Program

A continuing interest of the Segment is the development of the program on nitrosamines, since there are many indications of the formation of such materials in the environment. Several contracts in this area are being supported by the Segment. One at Thermo Electron Corporation (NO1-CP-45623) was largely responsible for aiding in the development of the thermo-luminous analyzer (TEA) which provides more sensitive and specific methods for analysis of various types of N-nitroso compounds found in the environment. At the University of Mississippi (NO1-CP-43347) the TEA has been used to develop analytical methods for nonvolatile N-nitroso compounds, such as nitroso amino acids which may occur in meat samples. In addition, this contractor has led the effort to couple high pressure liquid chromatography (HPLC) with the TEA and to develop an interface system from the HPLC to the TEA. This experience and information will be of inestimable value to the various laboratories using the TEA on a loan basis from NCI.

At the University of Oregon (NO1-CP-43348), by the use of the TEA, 3-hydroxy-N-nitrosopyrrolidine was identified both in fried bacon and in the fat from fried bacon. The British Food Manufacturing Industries Research Association (NO1-CP-44337) has developed a method for analysis of nonvolatile N-nitroso

compounds by denitrosation to produce nitric oxide which can be estimated in a chemiluminescent analyzer.

The project with Massachusetts Institute of Technology (NO1-CP-33315) continues to investigate the possible environmental formation of nitrosamines and the significance of the presence of precursors such as nitrite and nitrate. Endogenous nitrite seems to come largely from nitrate consumed in vegetables or cereals; bacterial and other systems reduce the nitrate to nitrite. Therefore, a microprocedure for nitrate has been developed to facilitate measurements in foodstuffs. From a survey in Colombia, it was determined that people from an area with a high gastric cancer incidence had very high nitrite levels in the gastric contents. Both bacteria and yeasts were found to enhance the formation of certain nitrosamines.

Mechanistic and Metabolic Studies

A project at Southern Research Institute (NO1-CP-55721) is concerned with the metabolism of aromatic amines and certain halogenated aliphatic compounds which were shown carcinogenic through the bioassay program. The halogenated compounds were bound to rat liver proteins through an inducible enzymatic reaction. Tentatively, it appears that didemethylation of 4,4'-bis(dimethylamino)benzophenone is a metabolic reaction. A related study at Fred Hutchinson Cancer Research Center (NO1-CP-44719) indicated that 4,4-bis(dimethylamino) benzophenone and the related methone base were metabolized to many compounds which were excreted in the urine. A contract at the American Health Foundation (NO1-CP-44639) showed that of all the identified urinary metabolites of o-toluidine, only 2-nitrosotoluene had mutagenic activity.

In a related project at Midwest Research Institute (NO1-CP-23270) a chromatographic method to separate model hydroxamic acids from urine or other biological fluids was developed. The technique will be extended to study hydroxamic acids derived from carcinogenic aromatic amines.

Still other projects supported by the Segment include one at Massachusetts Institute of Technology on the mechanism of action of aflatoxin and other mycotoxins from southeastern Asian foodstuffs (NO1-CP-43265). In another contract with M.I.T. (NO1-CP-33238), the effects of lipotropes in chemical carcinogenesis are being investigated. A diet marginal in lipotropes enhanced or depressed tumor induction depending on the model carcinogen. The project on lung adenoma induction in strain A mice at the University of California, San Diego (NO1-CP-33232) continues to provide a valuable screen for various environmental materials. Currently, water contaminants and certain dyestuff intermediates are being evaluated.

Studies in the *Chemistry and Molecular Carcinogenesis Segment* have been grouped into the following five major approach areas: (1) biochemical properties of chromatin and nucleic acids; (2) study of surface membranes and the manner in which they participate in the regulation of cellular properties; (3) study of polycyclic hydrocarbons and the significance to carcinogenesis of the many products which result from their enzymatic activation; (4) studies on the role of mutagenesis in carcinogenesis; and (5) study of the genetics of somatic cells, the chemically-induced mutations that can be induced in them, and the

significance of this process in carcinogenesis.

Earlier reports showing that the most important carcinogenic metabolite of polycyclic hydrocarbons is the 7,8-dihydro-9,10-epoxide have been further solidified and the mutagenicity of this compound evaluated in several systems. A similar mechanism has been shown to be operative for some other polycyclic hydrocarbons as well. The very close correlations observed among mutagenesis, carcinogenesis and DNA binding of these metabolites has rendered it highly likely that their mode of action depends upon binding to DNA. Unfortunately, budgetary restrictions have prevented the Segment from entering into additional experiments on DNA repair, but the importance of this process has been demonstrated in UV carcinogenesis in certain fish. Altered transport processes may be of crucial importance in malignant cells, especially those processes that concern transport of calcium, protons, and lactate. The mitochondrion is the chief organelle involved in these systems.

The enzymes responsible for activation of polycyclic hydrocarbons have been further characterized in human populations, but a test of the hypothesis that elevated values are present in lung cancer patients showed there was no such difference in enzyme inducibility.

Studies on the significance of mutagenesis in carcinogenic process have shown many similarities in the two processes. There are, however, as described below, some important quantitative differences both in extent and the time required for expression that suggest caution in accepting the hypothesis at its face value. Another mechanism, involving recombination of integrated viruses resulting from the action of carcinogens, is under study and may merit further consideration. Another type of mutation involving changes in nuclear non-histone proteins controlling transcription has also been pursued.

Biochemical Properties of Nucleic Acids and Chromatin

Studies at the Brookhaven National Laboratory (YO1-CP-50202) continue to reveal similarities in the damage done to DNA by ultraviolet radiation and chemical carcinogens. The small tropical fish, *Poecilia formosa*, which may be grown in clones, was used. It had been shown previously that thyroid cells after UV radiation and injection into recipient animals gave rise to thyroid tumors in the injected animals. Similar studies with gamma irradiation have also produced thyroid tumors. A comparison of the thyroid glands in closely related fish of this genus has led to the conclusion that the gland in *P. formosa* was in a pre-tumorous condition. Such fish appear to have a high probability of developing thyroid tumors.

Investigators at the Weizmann Institute (NO1-CP-33220) are concerned with the hypothesis that chemical carcinogens may enhance viral-induced cell transformation by influencing the recombination events which insert viral DNA into the host genome. They have established that there are preferred sites on the cellular genome where these events may occur and that recombination between integrated viral genomes and exogenous viral genomes can occur. In other experiments, they have demonstrated that a methylated form of guanosine inhibits some viral protein synthesis. Such a methylated product may arise as

a result of carcinogen reaction.

Investigators at Vanderbilt University School of Medicine (NO1-CP-65730) have continued their characterization of certain non-histone DNA-binding proteins from the nucleus. By immunological methods, it had been shown that those proteins isolated from malignant cells differed from similar proteins from normal cells. New methods were developed for the isolation of these proteins in larger amounts preparatory to characterizing them further. Chromatins from embryonic and regenerating liver were found to fix complement in the presence of Novikoff hepatoma antisera. Immunoadsorption experiments indicate the presence of at least two kinds of specific antibodies, one oriented against antigens common to highly proliferative tissues and the other oriented against antigens specific for malignant tumors.

Investigations at Polysciences, Inc. (NO1-CP-23245) on improved methodology for electrophoretic characterization of large proteins and nucleic acids have shown that, contrary to expectation, the pore size of the gel was not increased as the length of the cross linker was increased but that associative effects tended to produce smaller pores. In addition to indicating that previous theoretical calculations on pore size are probably invalid, it offers new insight into the mechanism by which size discrimination is achieved in such gels.

Surface Membranes

The properties of the cell membranes which control the passage of materials in and out of the cell and which regulate their intracellular distribution may play a significant role in the development of malignancy. The Segment has undertaken collaborative studies to obtain more information in this area.

Progress in studies of mammalian cell transport systems by Johns Hopkins University (NO1-CP-45610) is too extensive to summarize briefly. A hitherto unrecognized transport system carries lactate, the product of the rapid breakdown of glucose by tumor cells, out of the cell at a very high rate. This transport system is dependent upon the integrity of a particularly sensitive sulfhydryl group located near the lactate site and suggests that an affinity inhibitor of lactate transport can be developed. If the lactate transport system of tumor cells could be selectively interfered with, important changes in tumor cell metabolism might be achieved. The concentration of calcium in the cytosol of cells regulates many important intracellular activities; and it has been found that the concentration of calcium in the cytosol of tumor cells is much more tightly regulated than in normal cells, possibly at a much lower level. The transport of calcium across the plasma membrane and the mitochondrial membrane are the principal mechanisms by which the calcium level is regulated. Although several types of shuttle systems which transport electrons from NADH into the mitochondrial respiratory chain are known to occur in biologically normal cells, all but one were found to be absent in tumor cells. By newly-devised methods it has been demonstrated that at least three and possibly as many as four H^+ are ejected for each pair of electrons by the proton-pumping electron carriers in the mitochondrial membrane. These observations are of fundamental importance for understanding

of the mechanisms by which ATP is generated by mitochondria and have important reference to the regulation of acid base balance and hydrogen ion transport processes between the mitochondrial and cytosol compartments of cells.

Research at the Hebrew University (NO1-CP-43307) has examined transport mechanisms for uridine in quiescent and serum-stimulated cells and found that transport is rate-limiting in serum-stimulated cells, but phosphorylation is rate-limiting in quiescent cells. The latter limitation is overcome when serum stimulation activates the phosphorylation system. Similar differences characterize cells from golden hamster fibroblasts after they have been transformed by a murine sarcoma virus. In addition, a fluorescent label for the nucleoside transport system has been developed.

Two analogs of adenosine and one of phenylalanine have been studied at the University of Rochester (NO1-CP-45611). The chemical properties of these compounds have been investigated to provide clues as to the nature of the interaction. In addition, they have reported that normal hamster embryo cells and a line of polyoma-transformed cells derived therefrom differed in that certain phospholipids were decreased and others increased in the transformed cells. Other differences involving cholesterol and its esters were found in the membranes of these two cell systems. Whether these changes are related to transformation in a direct or indirect way is under investigation.

New electrophoretic techniques are being employed at the University of Manitoba (NO1-CP-43250) for the isolation and purification of human prolactin from amniotic fluid. It is hoped that new techniques will yield product comparable to that obtained from frozen pituitary glands. Approximately 90% of the total amino acid sequence of human pituitary prolactin has been determined by collaborators in this project. The laboratory continues to provide human prolactin and antisera to human prolactin to interested researchers. These antigens and antisera provide for radioimmunoassays and for studies of prolactin receptors and the binding of prolactin thereto.

Carcinogenic Properties of Polycyclic Hydrocarbons and their Derivatives

Several contractors have been working on the mechanism by which polycyclic hydrocarbons are converted into active carcinogenic metabolites and possible genetic differences in the way these metabolites are derived in human subjects.

At the Weizmann Institute (NO1-CP-02217) over 15 derivatives of benzo(a)pyrene have been tested to identify the mutagenic metabolites. The epoxides were by far the most active as mutagens and several of the isomers of the 7,8-dihydro-9,10-epoxide were further studied. In addition, it was reported by these workers that two distinguishable forms of the aryl hydrocarbon hydroxylase were under independent regulation in mammalian cells.

At the Institute for Cancer Research (NO1-CP-33367) further characterization of the diol-epoxide of benzo(a)pyrene was made. It was shown that the DNA reaction resulted from attack on the 2 amino group of guanine by the 10 position of the diol-epoxide. Analogous studies were performed with 7-methylbenzanthracene and 3-methylcholanthrene and it was concluded that the DNA reaction was mediated via the diol-epoxides in each case. The use of

radioactive-labeled carcinogens enabled the demonstration that the mutation rate generated by these compounds was closely related to the extent of DNA binding and has established that the *in vivo* reactions of 7-methylbenzanthracene and 3-methylcholanthrene are highly potent mutagenic agents. Studies in epithelial cells have established that the metabolic activation steps in epithelial cells are very similar to those in fibroblast cells.

At the University of California (YO1-CP-50203) studies have indicated that more than one form of aryl hydrocarbon hydroxylase is involved in the cytotoxic response of cells to benzo(a)pyrene. In cells which metabolize hydrocarbons there was a decreased average residence time in G1 and an increased residence time in G2+M. Spectral evidence indicates heterogeneity of the DNA benzopyrene adduct and electrophoretic and electron microscopic evidence has indicated nicking of DNA. This evidence has led to the postulate that the diol-epoxides form unstable phosphotriesters, subsequent hydrolysis of which cleaves the DNA chain.

Synthesis at the Ohio State University (NO1-CP-43248) of 10-methyl-benzo(a)-pyrene and 7,10-dimethyl-benzo(a)pyrene has permitted an evaluation of these compounds as carcinogens. These compounds should be non-carcinogenic if current thinking about the importance of the diol-epoxides is correct.

As part of their program to develop magnetic assays for cytochromes P-450 in liver and in liver microsomes at the Albert Einstein College of Medicine (NO1-CP-55606), a correlation between sex-linked steroid metabolism and particular magnetic resonance absorptions in rat liver microsomes has been demonstrated. In order to improve the magnetic assay for individual components of cytochromes P-450, a third harmonic detection approach has been developed as a means of improving the spectral resolution.

Based on an earlier study of twins at the Health Research, Inc. Roswell Park Memorial Institute (NO1-CP-55629), it was concluded that AHH inducibility was highly heritable. This property was utilized to assess the AHH inducibility in lung cancer patients indirectly by determining the inducibility in their progeny. In a carefully designed study there was no difference found between lung cancer patients and controls with respect to AHH inducibility. This result failed to confirm earlier reports of such an association.

At the University of Texas (NO1-CP-55604) studies on the factors affecting the reproducibility of the AHH assay were continued and several important factors identified.

In the studies at the University of Wisconsin (NO1-CP-65734) an excellent correlation was observed between the *in vitro* AHH test and the oxidative metabolism of several drugs *in vivo*. This study indicated that such techniques might be useful as an alternative to the AHH test and provided further evidence for the existence of three well-defined groups within the human population.

The Relationship between Mutagenesis and Carcinogenesis

Many recent reports have established beyond question that many carcinogens are mutagens and vice versa, but whether the carcinogenic process stems from the mutagenic process is less clear. Three contractors have dealt with this

problem. At Johns Hopkins University (NO1-CP-55713) a mammalian cellular system using hamster embryo cells was developed for the concomitant study of neoplastic transformation and somatic mutation. Factors important in the expression of the mutation by carcinogens were examined,and quantitative assays to measure changes in morphology, etc. as cells became malignant were also developed. The data indicate that neoplastic transformation differs from somatic mutation in the time required for expression, the frequency of the initial phenotypic changes, and the lack of homogeneity in the cellular response; the data further indicate that neoplastic transformation is a progressive process in which cells pass through many qualitatively different stages. Such a process might be initiated by mutational change, but cannot be completely described by a single event.

At the Weizmann Institute (NO1-CP-02217) similar studies showed the ratio of transformation to mutagenesis to be about 20 to 1 and indicated that transformation has a target equivalent to about 20 normal genes. There may thus be this number of the same or different genes, the mutation of any one of which by a single hit is required for transformation.

At the Wistar Institute (NO1-CP-55655) specific clones of 3T3 cells are under investigation and optimal conditions for selecting mutants and estimating transformation frequencies are being determined.

Somatic Cell Genetics

Recent progress in methodologies useful for studying and developing specific mutants of somatic cells provides a basis for optimism regarding the identification of specific mutations important in transformation, as well as explaining some cases of drug resistance in cancer patients. Work at the Ontario Cancer Institute (NO1-CP-43331) on the chromosome karyotype in Chinese hamster ovary cells indicates that, although this karyotype is quite abnormal, it is very stable. Chromosome segregation increases markedly in going from these quasidiploid cells to higher ploidies and on treatment with a variety of mutagens. In some cases segregation can be equated with chromosome loss. Conditional lethal temperature-sensitive mutants of CHO cells have been used as a means of shutting down protein synthesis in a manner which will allow the development of models for control processes and cell killing. The pleiotropic resistance to several drugs used as chemotherapeutic agents is similar to the pleiotropic phenotype of CHO colchicine-resistant cells. Some of these mutants have been characterized and found to contain a variety of mutations, including transport and altered enzymatic activity.

At Yale University (NO1-CP-55673) a technique has been perfected which makes it possible to transfer one or a few chromosomes from donor to recipient cells. Because the process permits the evaluation of the genetic role of individual chromosomes in a simple direct fashion, the procedure allows the identification of genetic factors which influence various cell processes. The activation of spermatid nuclei after fusion with HeLa cells has been found to be a workable way to begin production of haploid cell lines which should be of great use in both carcinogenesis and mutagenesis testing.

Contractors at the University of California (NO1-CP-43239) report the isolation of 5 additional mutant mouse cells defective in some phase of purine or pyrimidine nucleotide transport or synthesis. In addition, they have developed improved new techniques for the identification of the enzymes under study.

Isolation of additional mutants has been reported from New York University (NO1-CP-33279). Several of these are ts mutants which block in a specific portion of the cell cycle. On changing temperature, several become arrested in G1 and others at G2. Because chemically transformed cells, starved for serum, appear to stop at unique points in the cell cycle, these findings are of great interest.

CONTRACT NARRATIVES

CHEMISTRY OPERATIONAL UNIT

July 1, 1976 through September 30, 1977

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP-55606)

Title: Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Jack Peisach

Project Officers (NCI): Dr. Paul Okano
Dr. David G. Longfellow

Objective: To develop a magnetic assay for cytochrome P-450s isolated from mammalian liver microsomes, the cytochrome P-450 being the terminal oxidase involved in the aryl hydrocarbon hydroxylase enzyme complex. To achieve this goal, the technique of electron paramagnetic resonance was refined to obtain spectral resolution and thus to magnetically characterize five different cytochrome P-450s. A theoretical treatment of EPR data was performed so that structural information about low-spin ferric cytochrome P-450s could be ascertained. The assignment of structure for NO and CO cytochrome P-450 was made with model compound studies defining the mercaptide sulfur in this structure. The role of high-spin cytochrome P-450 in AHH activity was also studied.

The rate of the CO-inhibited reaction is of greater interest than the reaction in the absence of CO. CO inhibits the reaction by only 90-95%. It has been shown that with 5% CO, enough to bind to virtually all ferrous cytochrome P-450, microsomes from 3-methylcholanthrene treated animals reduce amaranth at twice the rate as microsomes from phenobarbital treated animals. It has been demonstrated that dye reduction proceeds only after CO is removed from the cytochrome. It is suggested then, that the apparent off-rate of CO is different in some or all of the population of cytochrome P-450 in 3-methylcholanthrene (3-MC) versus phenobarbital treated animals. With 1% CO, this difference cannot be observed, as CO does not bind completely to all the ferrous P-450 in microsomes from 3-MC induced animals. It is suggested that the P-450 with low affinity (high off-rate) for CO is causing the difference in the 5% CO case. A correlation is being made with AHH activity and amaranth reductase activity as a function of CO concentration in order to develop this as an assay system. The dye reduction activity is easily followed due to the large extinction coefficients of the components of the system and may, thus, be a marked improvement over standard AHH assay procedures.

As another phase of this study, an attempt is being made to understand the fundamental properties of the heme and its ligands as relate to oxidase activity. The back bonding of RS^- to the low-spin ferric iron has been calculated using an approach based on the symmetry properties of the heme-ligand

complex as measured with pulsed EPR spectroscopy. This same approach can be used to define symmetry properties of all paramagnetic materials.

In addition, pulsed EPR can be used to examine the interaction of the paramagnetic metal site of cytochrome P-450 with close-lying nuclei. This latter technique is sensitive only to nuclei with spin $I > 0$. In this way, specific interactions of the cytochrome with substrates and inhibitors can be directly ascertained.

Major Findings: Since the submission of the last fiscal report, further attempts have been made to develop a magnetic assay for cytochrome P-450s in liver and in liver microsomes which are the terminal oxidases in the aryl hydrocarbon hydroxylase enzyme complex. A correlation between sex-linked steroid metabolism and particular magnetic resonance absorptions in rat liver microsomes has been demonstrated. In order to improve the magnetic assay for individual components of cytochrome P-450s, a third harmonic detection approach has been developed as a means of improving the spectral resolution. Success has been primarily achieved in studies of nitrosyl hemoglobin where previous direct EPR has not allowed a resolution of the nine-line superhyperfine pattern observed in the EPR of single crystalline protein. This same approach is being presently employed using rat liver microsomes.

Heterogeneity of low spin heme protein components has been studied in an analogous system, cytochrome c, where heterogeneity is always observed, provided spectral resolution is achieved. For these proteins, magnetic, optical, and redox properties have been correlated to differences in heme-ligand structures. For cytochrome P-450s, these differences in physical properties are not believed to reside in the heme-ligand structures (which are thought to be constant for all P-450s) as much as in differences in the protein structure surrounding the prosthetic group.

As aryl hydrocarbon hydroxylase is related to the particular type(s) of cytochrome P-450 induced with 3-methylcholanthrene, as compared to phenobarbital, we have attempted to demonstrate differences in cytochrome P-450s based on differences of redox properties in microsomes. Using amaranth (Red dye 2) we have shown that azo reductase activity in microsomes is independent of induction procedure. All cytochrome P-450s, even from P. putida, reduce the dye the same way. The reductase activity is always dependent on P-450 concentration. The cytochrome P-450 dependence of the reaction was confirmed using the cytochrome P-450 inhibitor metyrapone and CO. The light reversal of the CO-inhibited reaction is wavelength dependent, being most efficient between 435 and 475 nm. Better wavelength resolution of the light is being developed at this time.

Significance to Biomedical Research and the Program of the Institute: These results facilitate the development of a nondegradative assay for AHH using a biophysical probe.

Proposed Course: This coming year, attempts will be made to correlate the magnetic findings with those for cytochrome P-450s in soluble preparations

with the hope that the EPR signature of the cytochrome P-450 associated with AHH can finally be defined.

Date Contract Initiated: August 6, 1974

Current Annual Level: $93,518

AMERICAN HEALTH FOUNDATION (NO1-CP-55639)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Directors: Dr. Emerich S. Fiala
Dr. Stephen S. Hecht

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives of this program are to clarify the metabolism and mechanism of action of the carcinogen o-toluidine. Having identified the major in vivo metabolites of this compound in the rat, hamster, mouse, and guinea pig during the first phase of this program, the problems now being addressed include a) the identification of minor metabolites produced in vivo, b) the nature of the metabolites produced during the incubation of selected tissues with o-toluidine or its derivaties in vitro, and c) the characterization of possible interactions of the metabolites with important cellular macromolecules both in vivo and in vitro. The results obtained will be significant not only in delineating the mode of action of the carcinogen o-toluidine, a major industrial product, but also in understanding the peculiar biological significance of the ortho-methylamine configuration in other arylamine carcinogens.

Major Findings: An economical synthesis of o-toluidine hydrochloride-methyl-^{14}C was developed. In rats, 0.31% of the administered dose of o-toluidine-^{14}C (100 mg/kg) appears in the exhaled air as $^{14}CO_2$, 75% appears in the urine, and 1.7% appears in the feces in 72 hours.

Paper electrophoresis, Sephadex LH-20 chromatography, high pressure liquid chromatography, and dextran ion-exchangers have been used for the detection and estimation of major and minor metabolites of o-toluidine-^{14}C in biological fluids. In the rat, the sulfate esters and the glucuronides of 4-amino-m-cresol and of N-acetyl-4-amino-m-cresol are the major urinary metabolites. Minor urinary metabolites are the glucuronide of anthranilic acid, free o-toluidine, o-aminobenzyl alcohol and N-acetyl-o-toluidine. o-Toluidine-N-glucuronide, the glucuronide of N-acetyl-4-amino-m-cresol, and the sulfates of N-acetyl-4-amino-m-cresol and of 4-amino-m-cresol are the major urinary metabolites in the guinea pig. The glucuronide of N-acetyl-4-amino-m-cresol and the sulfate of 4-amino-m-cresol and its N-acetyl derivative are the main urinary metabolites in the Syrian golden hamster. In the rat as well as the hamster, the glucuronide of anthranilic acid and, in trace amounts, o-nitrosotoluene were also detected. o-Nitrosotoluene was found mutagenic in

Salmonella strain TA-100 with or without S-9 activation. The rate of N-acetylation of o-toluidine in vitro using rat liver cytosol was 1/2 that of m- or p-toluidine.

Significance to Biomedical Research and the Program of the Institute: o-Toluidine, a major industrial chemical, produced annually on a tonnage basis, induces subcutaneous and urinary bladder tumors when fed to male rats and causes tumors of the spleen and liver in mice. Under identical conditions, m-toluidine is inactive and p-toluidine shows only marginal activity. Thus, metabolic studies designed to elucidate the activation and detoxication pathways of o-toluidine are relevant to mechanisms of carcinogenesis.

While there is now no evidence that human exposure to o-toluidine had led to adverse effects, including cancer, it is pertinent to point out that historically the bladder cancers originally seen by Rehn were named "Aniline" cancers. Although it cannot be exluded that these cancers were due to higher homologs, it is possible that o-toluidine may also have played a role.

Proposed Course: The metabolic studies proposed include the identification of minor metabolites of o-toluidine-^{14}C in the urines and biles of rats and other species such as the hamster, after either a single dose or during continued administration of the carcinogen in the diet. These studies will be supplemented by in vitro experiments in which ^{14}C labeled o-toluidine or its derivatives are incubated with homogenates, microsomes, and other subcellular fractions from various organs (liver, bladder mucosa, spleen) of rats and hamsters and the resulting labeled products are identified and quantitated. More extensive organ distributions, subcellular distributions, and determinations of in vivo binding to DNA, RNA, and selected proteins of target as well as non-target tissues will be undertaken. Isolation and identification of the monomer-carcinogen adducts from hydrolyzed biological macromolecules will yield yet another indication of the ultimate carcinogenic form.

The results of metabolic studies in vivo, studies of reactions with macromolecules in vivo and in vitro model studies, and mutagenicity data should give a strong indication of the ultimate carcinogen of o-toluidine. This substance will then be synthesized in quantities sufficient for bioassay and carcinogenicity studies will be undertaken.

Date Contract Initiated: June 30, 1975

Current Annual Level: $65,000

BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION (NO1-CP-44337)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. C. L. Walters

Project Officer (NCI): Dr. Larry K. Keefer

Objectives: To develop a method for the determination of total N-nitroso compounds directly on solid phase samples without the necessity for extraction and to determine the method's scope and limitations.

Major Findings: The volatile products of denitrosation of a non-volatile N-nitroso compound can be transferred in nitrogen through a converter, bringing about dehalogenation to produce nitric oxide for estimation in a modified chemiluminescent analyzer. In this manner, a linear chemiluminescent response has been obtained from N-nitrososarcosine over at least the range 5.7ng to 200µg. Positive responses are also given by inorganic nitrite and S-nitrosothiols, but these can be differentiated from that of N-nitrososarcosine by preliminary treatment with acetic acid followed by the denitrosation system of hydrogen bromide in the same solvent. The procedure has been evaluated for reliability, detection limit, etc. in synthetic media as well as certain commercial foodstuffs.

Significance to Biomedical Research and the Program of the Institute: Nitrate is widely distributed in the environment and is readily reduced to nitrite. Some nitrosatable amines present in biological systems yield N-nitroso derivatives which can be separated on the basis of their volatility or extractability while other such derivatives are extracted with extreme difficulty, if at all. Most N-nitroso derivatives are carcinogenic or can lead to such carcinogens by trans-nitrosation; thus, a method of determination of non-volatile compounds of this type would permit epidemiological studies on the basis of the exposure of different populations, in accordance with the objective of the Institute in pinpointing any relationship between environmental agents and cancer in man.

Proposed Course: The high blank values found for certain control samples will be studied; if their cause is found not to be due to natural nitrosamine contamination, means will be sought to eliminate the false positive response. The method will be compared with thermal energy analysis, another technique currently available for analyzing certain non-volatile N-nitroso compounds. Once the basic method is fully developed, applications to more complex foodstuffs such as meats will be explored, and values for the total N-nitroso compound content will be determined for a variety of environmental samples.

Date Contract Initiated: June 1, 1974

Current Annual Level: $56,766

CALIFORNIA, UNIVERSITY OF (at San Diego) (NO1-CP-33232)

Title: Study on Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay

Contractor's Project Directors: Dr. Michael B. Shimkin
Dr. Alexis J. Kniazeff
Dr. Jeffrey C. Theiss

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives of this project are twofold: (1) to test environmental compounds for carcinogenic activity using the strain A mouse pulmonary-tumor-induction-technique and (2) to evaluate the effect of virus infections on the chemical induction of pulmonary tumors in strain A mice.

Major Findings: (1) Bioassay - The strain A mouse lung tumor system was applied to the bioassay of a series of organic contaminants of U.S. drinking waters and of a group of halogenated compounds selected for mutagenicity-carcinogenicity comparison. The tumor response of chemically treated mice was compared to that of the appropriate controls with the following results: (a) Organic Water Contaminants - Of the 16 compounds tested, only bromoform was carcinogenic in mice under the conditions employed. A review of the literature suggested that chloroform, a known carcinogen (Report No. 1 NCI Carcinogenesis Technical Report Series, 1976) was negative in the pulmonary adenoma bioassay because kidney toxicity induced by chloroform in the male mice masked the carcinogenic effect of the compound. The fact that bromoform was positive in this system while chloroform was negative may be explained by the finding that brominated compounds are generally more active in the pulmonary adenoma bioassay than the corresponding chlorinated compounds (Cancer Res., 35: 1411-1415, 1975). While the pulmonary adenoma response to dichloromethane was not significantly greater than the response of vehicle-treated controls, the response was somewhat elevated at all three dose levels employed and the value obtained at one-fifth the maximum tolerated dose just missed significance. In view of the fact that dichloromethane is an alternative to trichloroethylene in the extraction of caffeine from coffee (Report No. 2. NCI Carcinogenesis Technical Report Series, 1976), a large scale test may be warranted. (b) Mutagenicity - Carcinogenicity Comparison of Halogenated Compounds - Of the 16 brominated compounds evaluated only 2-bromobutane was carcinogenic, although several of the compounds were mutagenic to bacteria (L. Poirier, Personal Communication). The lack of carcinogenicity of these mutagenically active compounds may be explained by their toxicity to strain A mice. It is thus possible that the general toxicity produced by the brominated compounds in this study masked any carcinogenic effects.

(2) Virus-Chemical Carcinogenesis - Results of several experiments performed to evaluate the effect of Reovirus infection on the pulmonary tumor response of strain A mice to chemical carcinogens may be summarized as follows: Single infection of mice with Reovirus either before, at the same time as, or after treatment of the mice with urethan resulted in a suppression of lung tumor formation, while multiple infection with Reovirus either had no effect on the lung tumor response to urethan or enhanced this response somewhat.

Significance to Biomedical Research and the Program of the Institute: The bioassay portion of this project integrates well into the activities of the Carcinogenesis Program by providing a resource for the testing of various compounds suspected of carcinogenic activity. In addition, structure-activity studies such as the carcinogenicity-mutagenicity comparison of halogenated compounds presently being conducted will hopefully provide information which will facilitate prediction of the carcinogenic potency of untested compounds.

The virus-chemical carcinogenesis studies have great value because these investigations are providing useful information concerning the role viral infection plays in the carcinogenic response to chemicals.

Proposed Course: (a) Bioassay - A study has recently been initiated to determine if chlorinated derivates of the brominated compounds tested in the mutagenesis-carcinogenesis study may be carcinogenic due to the decreased toxicity of these chlorinated compounds. Tests on several dyestuff intermediates and on additional organic drinking water contaminants will also be initiated shortly. A series of compounds which suppress the spontaneous incidence of lung tumors in mice will also be tested to see if they have a like effect on the response to known carcinogens. This study is designed to determine if some environmental compounds may actually be anticarcinogenic rather than carcinogenic.

(b) Virus-Chemical Carcinogenesis - An evaluation of the effect of Lactate Dehydrogenase Virus on the pulmonary tumor response to urethan is now in progress and a study of adenovirus effects on the carcinogenic response to urethan will be undertaken in the near future.

Date Contract Initiated: June 22, 1970

Current Annual Level: $148,933

CALIFORNIA, UNIVERSITY OF (at San Francisco) (NO1-CP-43239)

Title: Selection and Propagation of Somatic Cells Having Specific Physiological Mutations

Contractor's Project Director: Dr. David W. Martin, Jr.

Project Officer (NCI): Dr. John Bader

Objective: To provide a series of thermosensitive regulatory mutants in which specific gene products responsible for control of purine and pyrimidine nucleotide synthesis, and thus cellular growth and division, have been genetically altered.

Major Findings: A clone (AE_1) of mouse lymphosarcoma cells having a markedly impaired ability to transport all purine and pyrimidine ribo and deoxyribonucleosides has been isolated. These mutant cells possess the enzymes required for the phosphorylation or phosphorolysis of nucleosides, and are capable of transporting adenine, guanine, and hypoxanthine normally. The kinetic analysis of the uptake of purine and pyrimidine nucleosides in wild type and AE_1 cells provides further evidence that the transport of purine and pyrimidine nucleosides is mediated by a common carrier. Attempts are currently underway to select temperature-sensitive revertants of these transport defective mutant cells. Such conditional transport mutants will provide powerful tools for a pharmacological analysis of chemotherapeutic agents which are analogs of purine or pyrimidine nucleosides. Azauridine-resistant mouse lymphosarcoma

cell mutants have been isolated. Azauridine is known to be phosphorylated by uridine kinase to 6-Aza UMP, a competitive inhibitor of orotidylate decarboxylase, the ultimate enzyme in the pathway of de novo pyrimidine nucleotide biosynthesis. The initial characterizations of three independently-derived azauridine-resistant mutants revealed that all possessed 3 to 8-fold increases in intracellular PPriboseP, an important common precursor of nucleotide biosynthesis, and all three have a 20-30% increase in intracellular ATP concentrations. One mutant also appears to have an altered PPriboseP synthetase enzyme. A mutant clone which is resistant to the combination of hadacidin, adenosine, uridine, and erythro 9 (2-hydroxy-3-nodal) adenine, an inhibitor of adenosine deaminase, has been isolated. The mutant is sensitive to the toxic effects of 6-mercaptopurine but grows at a normal rate and possesses normal catalytic activities and electrophoretic mobilities of the purine salvage enzymes. These mutant cells contain diminished intracellular concentrations of PPriboseP and possess reduced rates of de novo purine synthesis in the face of normal growth rates. Clones of S49 cells resistant to 0.5 mM hydroxyurea, a known inhibitor of DNA synthesis at the level of ribonucleotide reduction, have been isolated. The intracellular nucleotide concentrations, the catalytic activity, and isoelectric points of purine salvage enzymes appear unchanged. Phosphonoacetyl L-aspartate (PALA) is a transition state analog of carbamylaspartate, and studies with PALA-resistant Chinese hamster cells demonstrate that the regulation of de novo pyrimidine nucleotide synthesis is not restricted to the early portion of the de novo pathway.

New analytical techniques for mutant characterization involving an isoelectric focusing technique for the detection of small quantities of the enzymes of purine and pyrimidine salvage have been developed. Using this technique, it is currently possible to detect hypoxanthine-guanine phosphoribosyl transferase, adenine phosphoribosyl transferase, adenosine kinase, thymidine kinase, purine nucleoside phosphorylase, adenosine deaminase, and PPriboseP synthetase, all at the sensitivity of 1% of wild type activity. Several of the salvage enzymes, particularly HGPRT, APRT, adenosine kinase, and purine nucleoside phosphorylase can be monitored simultaneously on the same slab gel for variations in activity and isoelectric points. Such provides an effective means of screening mutants with altered metabolism of purine and pyrimidine nucleotides to eliminate those with disordered salvage systems.

Significance to Biomedical Research and the Program of the Institute: The regulation of purine and pyrimidine nucleotide biosynthesis is closely aligned with the regulation of cellular growth and division as a major controlling element in nucleic acid synthesis under physiologic and pathologic conditions and during pharmacologic intervention.

Proposed Course: Temperature-sensitive revertants of these various mutant cells will be selected and isolated and molecular characterization will be completed for those that are currently not defined. The temperature-sensitive, conditional mutants will be of considerable benefit to investigators attempting to control purine and pyrimidine nucleotide synthesis, and thereby growth

and division, without the use of chemical inhibitors. The mutants will also provide cells in which the intracellular concentrations of various purine and pyrimidine nucleotides can be altered by growth of the mutants at permissive and restrictive temperatures.

Date Contract Initiated: March 24, 1974

Current Annual Level: $116,942

ERDA/NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) (Y01-CP-50202)

Title: Repair Mechanisms in Carcinogenesis

Contractor's Project Director: Dr. R. B. Setlow

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To obtain quantitative relations between damages to DNA resulting from chemical and physical agents and neoplastic transformation.

Major Findings: The small tropical fish Poecilia formosa may be grown in clones and, hence, cells from one animal may be injected into another without danger of immunological rejection. Analysis has been completed of a second experiment in which fish cells (mainly thyroid ones) are exposed to UV radiation in vitro and injected into recipient animals. The results confirm those of the first experiment in which such fish developed thyroid tumors, detected by gross pathology and histology, 6-9 months after injection. If the UV irradiation was followed by visible light illumination before injection (photoreactivation) no tumors developed, whereas visible light had no effect on tumor incidence if given before UV.

Analysis of animals injected with gamma-irradiated cells has also been completed. Fish injected with 400,000 cells exposed to 250 rad all had thyroid tumors and all showed extensive hemorrhages throughout the body. When cells exposed to 500 rads were used, only 72% of the animals had tumors and only 60% were hemorrhagic. The sensitivity of the above responses indicated that the thyroid gland in P. formosa may differ from that typically found in fish. Comparisons were made between the thyroid of P. formosa and of five closely related Poiciliidae, which were kept under the same environmental laboratory conditions. In the five related species, the thyroid gland was composed of few uniform rounded follicles, lined with low to cuboidal epithelial cells, surrounding abundant colloid. In marked contrast, the thyroid gland P. formosa consisted of large areas of microfollicles and afollicular groups of epithelial cells. The follicular cells were hypertrophied and in some areas of the gland there was hyperplasia of the connective tissue. The appearance of the gland was similar to that seen during early stages of the development of thyroid tumors in highly inbred strains of fish. It was concluded that the gland of P. formosa was in a pre-tumorous condition. Young P. formosa appear to have a high probability of developing thyroid tumors under the influence of carcinogens or transformed cells, yet at this early stage the

response is quite precise and specific. Experiments have been started using UV-irradiated liver and spleen cells injected into the abdominal cavity and using UV-irradiated thyroid cells injected into the tails of recipient animals.

Significance to Biomedical Research and the Program of the Institute: These data permit an identification of the sensitive molecules and the nature of the lesions in them. Hence one will be able to estimate quantitatively the probability of a transformation per individual chemical change in DNA and, therefore, the quantitative hazards from chemical carcinogens.

Proposed Course: UV experiments using cells from liver and spleen will be completed, and the UV experiments on thyroid cells will be repeated, using different UV wavelengths so as to obtain the action spectrum for tumor induction. Ionizing radiation experiments will be continued using irradiated cells other than thyroid, in an attempt to understand the hemorrhagic response observed earlier. Normal and tumor tissue will be transplanted from one clone to another to see how well such tissues grow in nonisogenic animals. Earlier experiments with chemical carcinogens such as N-acetoxy-AAF and others known to interact with DNA will be repeated. It is planned to use 4-Nitroquinoline oxide which has attributes, as judged by repair, of both UV and ionizing radiation and methyl methanesulfonate, which has attributes only of the ionizing radiation type. Attempts will also be made to repeat the experiments above with fish cells in culture and to score for transformation by loss in contact inhibition and possible neoplastic growth in vivo.

Date Contract Initiated: June 1, 1975

Current Annual Level: $77,200

ERDA/NCI INTERAGENCY AGREEMENT (University of California at Berkeley) (YO1-CP-50203)

Title: Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons

Contractor's Project Director: Dr. Melvin Calvin

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: To define the chemically reactive positions of polycyclic hydrocarbons and to determine the interaction of the reactive intermediates with cell macromolecules and the nature of interaction with oncogenic viruses.

Major Findings: Investigation of the response of mouse liver epithelial cells growing in culture to benzo[a]pyrene (BaP) has been continued. A comparison of the level of aryl hydrocarbon hydroxylase in clones of mouse liver epithelial cells (NMuLi) which had different sensitivities to the cytotoxic action of BaP, revealed a good correlation between cytotoxicity and

enzyme levels. Furthermore, sensitivity to the different metabolites and detailed studies of the enzymology of BaP metabolism indicated that more than one form of aryl hydrocarbon hydroxylase (AHH) was involved in the cytotoxic response.

In order to more fully characterize the effect of BaP on the cell cycle, studies have been made on the effect of the parent compound and various metabolic derivatives on the growth of a mouse epithelial cell line and a related clone that is resistant to the cytotoxic action of BaP. The resistant population lacks inducible AHH and its growth rate is only mildly affected by the parent hydrocarbon. In the sensitive cell line, both the parent hydrocarbon and 7,8-dihydroxy-7,8,9,10-tetra-hydrobenzo[a]pyrene 9,10-oxide decreased the average residence time in G_1 and increased the residence time in G_2+M. These effects were dependent on the ability of cells to metabolize BaP, since they were not evident in cells lacking AHH.

Efforts have been continued to establish a system to study the physical properties of the cytochromes of the AHH system. Selection was made of a mouse liver cell strain, NMuLi, for study because it had the highest AHH activity of any of our cell strains. The study of NMuLi microsomes by difference spectroscopy was complicated by the presence of light-scattering membrane fragments. It was concluded that spectrophotometric studies are not feasible using NMuLi cells. The use of primary liver cell cultures was begun, but more developmental effort is needed to increase the length of time the AHH levels will remain high and to demonstrate induction of activity with hydrocarbons.

Mainly through spectroscopic means it was demonstrated that the hydrocarbon structure of the carcinogen bound to a model nucleic acid, poly (G), was a derivative of 7,8-dihydroxy-7,8,9,10-tetrahydroBaP. Subsequently, a number of studies have established that the activated intermediate in binding to poly (G), a presumable DNA, is 7β,8α-dihydroxy-9α-10α-oxy-7,8,9,10-tetrahydroBaP (anti-diol epoxide). Recent focus has been on covalent adducts formed between DNA and enzyme activated BaP or synthetic anti-diol epoxide. Analysis shows at least two adducts in the case of microsomal activated BaP and eight from reacting the anti-diol epoxide with DNA. Fluorescence and co-chromatography data demonstrate that the enzyme-catalyzed adducts result from metabolism of the 7,8 and 9,10-double bonds of the hydrocarbon and suggest that the activated intermediate is diol epoxide.

It has been shown that the syn and anti-benzo(a)pyrene diol epo ides elicit a concentration dependent nicking of superhelical ColE1 DNA in an in vitro reaction monitored by agarose gel electrophoresis and electron microscopy. Since the kinetics of nicking appear too rapid for depurination strand scission, it was postulated that the diol epoxides form unstable phosphotriesters, hydrolysis of which nick the DNA.

Significance to Biomedical Research and the Program of the Institute: The widespread distribution of polycyclic hydrocarbons as a result of the combustion of fossil fuel, both in moving and in stationary power plants, as well as in the combustion of agricultural wastes, makes them important environmental carcinogens.

Their presence as a principal component of tobacco smoke whose carcinogenicity has been clearly demonstrated epidemiologically makes it even more critical to know their mode of action--the role of enzymes in the activation as well as in the binding of the carcinogen to cellular components and the components of oncogenic viruses. A first step has already been taken in helping to understand how these hydrocarbons may become active.

Proposed Course: To investigate the metabolic activation of polycyclic aromatic hydrocarbons and the subsequent transformation of mammalian cells. The research is interdisciplinary in nature and, consequently, approaches a similar problem from a number of different directions.

Date Contract Initiated: April 12, 1971

Current Annual Level: $89,559

FRED HUTCHINSON CANCER RESEARCH CENTER OF PACIFIC NORTHWEST RESEARCH (NO1-CP-55719)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Director: Dr. John D. Scribner

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: To identify the urinary metabolites of bis[4-(dimethylamino) phenyl]-methanone (Michler's ketone) and 4,4'-methylene-bis(N,N-dimethyl)-benzenamine (methane base), the tissue distribution of these carcinogens, and the extent of binding to tissue macromolecules.

Major Findings: Thin layer chromatography has demonstrated the presence of at least 13 different metabolites of methane base and of seven metabolites of Michler's ketone. Most of the radioactivity (from tritiated compounds) is found in two highly polar fractions from the methane base (45% of total), while 75% of the activity from Michler's ketone is found in a single TLC spot somewhat more polar than methylene bis(acetanilide). This spot can be further separated by high pressure liquid chromatography into two fractions, of which the major component has a UV spectrum indicating loss of the keto function. Gradient HPLC shows the presence of at least eight metabolites of Michler's ketone, while chromatography on Sephadex LH-20 shows nine different materials. Column chromatographic separation of the methane base metabolites is now under development.

Significance to Biomedical Research and the Program of the Institute: Identification of the metabolites of these compounds and the mode of their binding to macromolecules will give a better understanding of the mechanism of metabolic activation of environmental carcinogens. This should permit more useful methods of prescreening future potential carcinogens and a better

knowledge of the extent to which screening results (including whole animal testing) can be extrapolated to human risk.

Proposed Course: The progression of urinary metabolism with time will be monitored by HPLC. Various metabolites will be isolated and structure elucidation studies performed. The level of binding of radioactive metabolites to DNA, RNA, and protein of various tissues will be determined, using previously developed methodology.

Date Contract Initiated: July 1, 1975

Current Annual Level: $34,100

HEALTH RESEARCH, INC./ROSWELL PARK MEMORIAL INSTITUTE (NO1-CP-55629)

Title: Aryl Hydrocarbon Hydroxylase in Human Lymphocytes and the Relationship to Chemical Carcinogenesis

Contractor's Project Director: Dr. Beverly Paigen

Project Officers (NCI): Dr. Paul Okano
Dr. David Longfellow

Objectives: To confirm that aryl hydrocarbon hydroxylase (AHH) inducibility is genetically determined and that persons with higher AHH inducibilities have a higher risk of lung cancer.

Major Findings: Aryl hydrocarbon hydroxylase (AHH) inducibility was found to be highly heritable in a study of 48 pairs of monozygotic and dizygotic twins. Moreover, the data from the twin study suggests that AHH inducibility is more likely determined by a single gene.

AHH inducibility cannot be determined in a population of lung cancer patients because the lymphocytes of over half of these patients do not give readily detectable levels of AHH. The low AHH activity is due in part to reduced mitogen response. Thus, AHH inducibility in lung cancer patients was tested indirectly by determining AHH inducibility in the progeny of lung cancer patients and in an appropriately matched control population. An analysis of 57 progeny and 57 matched controls showed no differences between the two populations in basal AHH activity, induced AHH activity, or AHH inducibility. Thus, if lung cancer patients do have a distribution of AHH inducibility that is shifted toward the high end of the range, this is not passed on to their offspring.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons, an important class of environmental carcinogens, are found in cigarette smoke, polluted air, and food. These carcinogens are metabolized primarily by microsomal mixed-function oxygenases. If people vary in their ability to metabolize carcinogens, if this variation is

genetically determined, and if the variation affects risk to lung cancer, then several pathways are opened for cancer control. High-risk individuals could be identified and advised to give up smoking, to avoid high-risk occupations, and to seek regular medical checkups.

Proposed Course: To analyze further steps in the metabolism of polycyclic aromatic hydrocarbons to determine if there is genetic variation that alters risk to cancer.

Date Contract Initiated: August 12, 1974

Current Annual Level: $75,721

HEBREW UNIVERSITY (NO1-CP-43307)

Title: Mammalian Cell Transport Systems

Contractor's Project Director: Dr. Wilfred D. Stein

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: (1) To obtain reliable data and kinetic parameters for the uptake of cell nutrients and non-metabolizable analogues of these nutrients for a number of different normal cell lines and from the malignant cells derived from these, following viral or chemically-induced transformation. (2) To study the time course of the new transport kinetics following cell transformation to the malignant state. (3) To evaluate the influence of cell density on transport properties to determine the contribution of cell-cell interactions.

Major Findings: The field of uptake studies in cells in cell culture has been put on a firmer footing by accomplishing a careful dissection of the overall process of nutrient uptake into the two sequential processes of transport and subsequent metabolism. A full kinetic analysis has been worked out and various experimental criteria which enable a firm distinction to be made between these two processes has been set up. Work on the uridine transport system of 3T3 cells in the quiescent and growing state had established this as a well-controlled object for the analysis of the early events of growth stimulation. It now appears that for both quiescent and serum-stimulated cells a facilitated diffusion system is present, apparently equally effective in these two states, while uridine uptake proceeds by trapping of such entered uridine by a process which is now serum-stimulated. Transport is rate-limiting in serum-stimulated cells but it is phosphorylation that is rate-limiting in quiescent cells; this limitation being overcome when serum-stimulation activates the phosphorylation system. In the Nil 8 line of golden hamster fibroblasts, transformed by a murine sarcoma virus (the Nil 8 HSV strain), a distinction can also be made between transport and the subsequent phosphorylation if transport measurements are performed at short time intervals and, in addition, the subsequent rate of trapping of nucleoside is followed over a substantial period of time, at different concentrations of external uridine. By comparing these time courses of uptake with those of the

nucleoside cytosine arabinoside (which can be shown to be insignificantly phosphorylated), kinetic data have been derived on the facilitated diffusion system for the two nucleosides and for the in vivo phosphorylation of uridine. A parallel study was performed on the Rat B77 line of RSV-transformed rat primaries. (2) The analysis of the transport process itself has been taken further by developing a fluorescent label for the nucleoside transport system. The compound, S-(2-dansylaminoethyl)-guanosine (DAMG) has been prepared and was fluorescent with maximum emission bands at 400 and 550 nm. Kinetic studies showed that the DAMG inhibits uridine uptake by red blood cells with K_i in the range of 0.2-0.6 μM, and for uridine uptake by the Nil 8 cells the K_i was in the range of 3-5 μM. The peak at 400 nm can serve as a measure for the amount of DAMG present, while the peak at 350 nm gives information on the micro-environment of the probe molecule. It appears that the DAMG is specifically bound in or near the uridine transport site. We must now extend this work to studies on cells in culture.

Significance to Biomedical Research and the Program of the Institute: The events studied are closely associated with the early events of the cessation of cell growth following confluence and the reactivation of growth on stimulation. They are, hence, directly associated with the question of growth control, the key problem of cancer. An understanding of the processes concerned in the switching on and off of membrane transport must be basic to the understanding of growth control and its malfunction.

Proposed Course: (1) To carry out a survey of the uridine uptake system among the many cases of normal cell lines and their malignant counterparts, in an endeavor to find out whether it is the transport of uridine that is activated when cells transform to the malignant state or whether it is the subsequent metabolic trapping of uridine that is so affected. For each cell line, estimates of the kinetic parameters of transport and of the subsequent metabolism will be separately obtained, to yield a quantitative comparison between normal and transformed states. (2) To extend this analysis to the uptake of other metabolites such as the sugar transport systems and the ion transport systems. (3) To continue with the task of labelling, counting, and isolating the membrane transport systems, using those probes for the nucleoside systems that have been developed in the course of this work. These probes, and especially the fluorescent derivatives, will give information as to the state of the membrane in normal and transformed cells and thus lead to an understanding of the total picture of the transformations leading to cell malignancy.

Date Contract Initiated: June 25, 1974

Current Annual Level: $92,330

INSTITUTE OF CANCER RESEARCH/CHESTER BEATTY RESEARCH INSTITUTE (NO1-CP-33367)

Title: Nature of the Polycyclic Hydrocarbon-Nucleic Acid Compound in Hydrocarbon Carcinogenesis

Contractor's Project Director: Dr. Peter Brookes

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To understand the chemistry of the reaction between carcinogenic hydrocarbons and the DNA of mammalian cells which results when these compounds are metabolized by the cells. Furthermore, since it now seems probable that more than one reaction product is present in the DNA, the significance of each reaction will be considered in relation to mutagenesis and transformation.

Major Findings: The addition of borate buffer to the eluting solvent allowed the separation of LH20 Sephadex of the products of reaction of anti- from those of syn- BP- diolepoxide. It was therefore possible to show that the product which reacted with DNA in vivo following exposure of cells to BP was predominantly the anti-isomer, viz., 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, although some product of reaction of the syn-isomer was also present. A study of the chemistry of reaction of the anti-isomer with DNA showed the major product to result from attack on the 2-amino-group of guanine via the 10-position of BP. A minor product of reaction was shown to be an adenine derivative, probably 6-amino-substituted.

DNA from cells treated with 7MBA or 3MC was degraded enzymically and the hydrocarbon-deoxyribonucleoside products isolated using LH20 Sephadex. The fluorescence spectra of these products on comparison with appropriate synthetic standards has led to the suggestion that in the case of 7MBA, DNA reaction was mediated via the 3,4-diol-1,2-epoxide, while for 3MC the 9,10-diol-7,8-epoxide was the reactive intermediate.

The very high mutagenic efficiency of anti-BP-diol-epoxide towards both bacteria and mammalian cells has been confirmed by the use of tritium labeled material, allowing mutation induction to be related to the extent of DNA modification. The use of a cell-mediated mutation system has established that the in vivo reactions of 7MBA and 3MC, like that of BP, are highly potent mutagenic events.

Mouse salivary gland epithelial cell cultures have been used to establish that the metabolic activation steps leading to BP-DNA reaction are the same in epithelial cells as in the fibroblast cell systems normally used for such studies.

Significance to Biomedical Reasearch and the Program of the Institute: The chemistry of the in vivo reaction of one carcinogenic polycyclic hydrocarbon, namely BP, is now understood in considerable detail. The biological consequences of this reaction are fully consistent with the concept of the relationship between mutagenesis and carcinogenesis and support the use of mutation systems for the rapid screening of environmental carcinogens. The knowledge obtained for BP-DNA reaction must speed the investigation of other hydrocarbon carcinogens and suggest approaches to an understanding of the molecular biological basis of the origin of the cancer cell.

Proposed Course: Studies with 7MBA and 3MC will aim at a complete understanding of the nature of their in vivo DNA reaction. The molecular biological consequences of the carcinogen-DNA reactions will be investigated with the aim of explaining the basis of their profound biological consequences.

Date Contraction Initiated: June 25, 1973

Current Annual Level: $90,000

JOHNS HOPKINS UNIVERSITY (NO1-CP-55713)

Title: The Significance to Mutagenesis in Carcinogenesis

Contractor's Project Directors: Dr. Paul O. P. Ts'o
Dr. Carl Barrett

Project Officers (NCI): Dr. Rufus Day
Dr. Andrew C. Peacock

Objectives: (1) To develop the necessary techniques and an understanding of the in vitro neoplastic transformation system and somatic mutation system, employing Syrian embryonic fibroblasts. (2) To study the mutagenic and the transformation effects of MNNG, benzo(a)pyrene (BP) and its metabolites, and the incorporation of 5-bromodeoxyuridine into DNA followed by near UV irradiation. (3) To investigate the relationship between neoplastic transformation and somatic mutation. (4) To investigate the roles of genetic and epigenetic components in the processes of both neoplastic transformation and somatic mutation.

Major Findings: A Syrian hamster embryo fibroblast system was developed for the concomitant study of neoplastic transformation and somatic mutation. Following exposure to chemical carcinogens, morphological transformation of hamster embryo cells was measured. Chemically induced somatic mutation of the cells was assayed at two genetic loci. Mutants deficient in hypoxanthine phosphoribosyl transferase (HPRT) were detected by the production of colonies resistant to 8-azaguanine (AG^r) or 6-thioguanine (TG^r) and mutants with an altered Na^+/K^+ ATPase were detected by the production of colonies resistant to ouabain (Oua^r).

Factors involved in the quantitation of the mutation frequencies of hamster embryo cells following exposure to carcinogens were determined. The Recovery efficiencies of mutant cells were measured and the degree of cross feeding effects to $HPRT^-$ cells was determined. The expression time of the mutations was also examined. The mutation frequencies at the two loci of hamster embryo cells following exposure to MNNG or benzo(a)pyrene were determined. A comparison between the mutation frequencies of the normal hamster embryo cells and a subdiploid hamster cell line derived from the normal cells was made. Normal and transformed cells were found to have similar spontaneous and induced mutation frequencies to TG^r and Oua^r resistance, while the frequency of AG^r cells was significantly higher using transformed cells.

The progressive nature of neoplastic transformation was examined by studying the temporal acquisition of various in vitro phenotypes by Syrian hamster embryo cells. Such phenotypes included enhanced fibrinolytic activity and ability to grow in soft agar. Morphological transformation and enhanced fibrinolytic activity were early changes observed after treatment of the cells with benzo(a)pyrene, while the ability to grow in soft agar was delayed until 30-60 population doublings after treatment. The development of soft agar growth potential was related to the carcinogen treatment, since spontaneous transformation was very rare. The results indicate that cellular transformation is a phenomenon progressing through differentiable stages. Assuming that morphological transformation represents the initiation of the transformation process, a quantitative comparison between morphological transformation and somatic mutation at the two genetic loci was made. Both mutation and transformation were detected at 4-8 population doublings after treatment. The frequency of morphological transformation was approximately one hundredfold higher than somatic mutation. On the other hand, the frequency of cells capable of growth in soft agar was comparable to the frequency of somatic mutation, but the expression of this alteration required more than 40 population doublings.

Significance to Biomedical Research and the Program of the Institute: This project concerns three aspects related to research and control of cancer: (1) It provides basic knowledge and techniques for the establishment of in vitro neoplastic transformation assay and somatic mutation assay which may be useful in the detection and investigation of environmental and industrial carcinogens and mutagens. (2) This project will provide the basic understanding concerning the structure and function of the genetic apparatus as related to somatic mutation and to malignant transformation. Such a basic understanding will not only help in the design of an effective assay system for carcinogens/mutagens but will also provide information to be used in designing diagnostic techniques and therapeutic procedures for cancer. (3) While the study is being conducted at the cellular level, it will be related to the process of differentiation and, therefore, will provide a foundation for the investigation of carcinogenesis and cancer biology at the level of tissues and organelles.

Proposed Course: To define the in vitro neoplastic transformation and somatic mutation studies in much greater detail, particularly with regard to: (1) The progressive nature of the processes, and (2) the reversal of these processes.

To define the epigenetic and genetic nature of somatic mutation and neoplastic transformation processes by application of a genetic perturbation (BudR incorporation followed by near UV irradiation) and by a genetic manipulation (somatic hybridization).

To define the molecular nature of these neoplastic transformation and somatic mutation processes in karyotypic studies (including in situ hybridization) and by the application of techniques of molecular biology (such as nucleic acid hybridization, in vitro transcription, and translation, etc.).

Date Contract Initiated: June 27, 1975

Current Annual Level: $144,325

JOHNS HOPKINS UNIVERSITY (NO1-CP-45610)

Title: Studies of Mammalian Cell Transport Systems

Contractor's Project Director: Dr. Albert L. Lehninger

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: It is a general objective to examine specific transport systems of the mitochondrial membranes and plasma membranes of cancer cells and to determine in what ways they differ from those of normal cells. Particular attention was focussed on the membrane transport of energy-furnishing metabolites, H^+, Pi, and Ca^{2+}, between the mitochondrial and extramitochondrial cell compartments, as they relate to the regulation of the energy metabolism and the acid-base balance of the intracellular compartments of cancer cells and normal cells. Moreover, to ascertain to what extent these membrane changes are induced by oncogenic agents and to what extent they may be reversed.

Major Findings: Recent research in this laboratory has demonstrated the presence of a hitherto unrecognized lactic acid transport system in the plasma membrane of a number of ascites tumor cells. This system carries lactate, the product of the rapid breakdown of glucose by tumor cells, out of the cell at a very high rate. It has long been known that tumor cells show a highly accelerated breakdown of glucose to lactate, which pours into the blood and causes local acidification. The lactate transport system transports only a few normal metabolites, in particular, lactate and pyruvate. It is inhibited by certain analogs of lactate, namely α-cyanohydroxycinnamate and hydroxyphenyllactate, and promotes the equimolar exchange of lactate and OH^- across the membrane. The direction and rate of transport of lactate across the cell membrane depends on the difference in hydrogen ion concentration across the cell membrane. The lactate transport system, presumably a membrane protein(s), is inhibited in an unusual way by only certain sulfhydryl group reagents, indicating that the sensitive sulfhydryl group is located close to or at the lactate site. This observation suggests that an affinity inhibitor of lactate transport can be developed.

The transport of Ca^{2+} across the plasma membrane and the mitochondrial membranes of a number of tumors has been studied in some detail. These processes are highly relevant to tumor cell biology because the cytoplasmic Ca^{2+} ion concentration regulates many important intracellular activities in all types of eukaryotic cells. These studies have shown that in Ehrlich ascites tumor cells the rate of Ca^{2+} influx is very low. However, the mitochondria from this and other tumors show not only very high rates of inward transport but also remarkably tenacious retention of Ca^{2+} and phosphate. From these and other points of evidence it appears that the Ca^{2+} concentration in the cytosol

of tumor cells is more tightly regulated than in normal cells, possibly at a much lower level, and may thus poise the activity of a variety of different Ca^{2+}-dependent processes in tumor cells at a different level than in normal cells. In the past few weeks an important new finding has been made which may give important clues as to the nature of the biochemical signals that promote Ca^{2+} release from tumor mitochondria into the cytosol. The most important triggering event is a shift in the oxidation-reduction state of mitochondrial NAD to a more oxidized poise, as revealed by double-beam spectrophotometry.

With a newly perfected method of measurement, it has been determined that the transport of electrons from NADH, generated in the cytosol by glycolysis in tumor cells, across the mitochondrial membrane into the respiratory chain, takes place exclusively by the malate-asparate shuttle system in the six lines of tumor cells tested, which also operates in normal liver and heart. The other types of NADH shuttles known to occur biologically in normal cells appear to be essentially absent in the six different ascites tumors examined. These observations, which settle a long-standing question, also provide valuable evidence on the rate and function of the mitochondrial membrane transport systems for aspartate, glutamate, malate, and α-ketoglutarate. They have also permitted construction of detailed metabolic flux diagrams for five different lines of ascites tumor cells, which give, for the first time, the approximate rates of each enzymatic and transport step in glycolysis, the tricarboxylic acid cycle, and the NADH shuttle in these cells.

During the past year the most fundamental and important discovery made in this laboratory has come from more precise and unambiguous measurements of the number of H^+ ions ejected from mitochondria into the surrounding medium as a pair of electrons from respiratory substrates passes through each of the three energy-conserving sites of the respiratory chain. For some ten years it has been widely thought that $2H^+$ are ejected at each site at the expense of electron transport energy, as originally postulated by Mitchell in his chemiosmotic hypothesis. However, for some time it has been suspected in this laboratory that this stoichiometry is too low. With three newly devised methods, it has now been demonstrated that at least three and possibly as many as four H^+ are ejected per energy-conserving site by the proton-pumping electron carriers in the mitochondrial membrane. Moreover, by improved procedures, it has also been established that three H^+ are ejected per ATP hydrolyzed. These newer values for the proton stoichiometry are of fundamental importance with relation to the mechanisms by which ATP is generated by mitochondria, the power plants of the cell. They have evoked a basic modification of the chemiosmotic hypothesis of energy coupling. Moreover, the new data are also very important in the regulation of the acid-base balance and H^+ transport processes between the mitochondrial and cytosol compartments of cells. Although these observations were made on mitochondria from normal cells, they were first stimulated by consideration of some data on tumor mitochondria. Very recently, mitochondria isolated from Ehrlich tumor cells have been tested, with the finding that they very likely have idential H^+/site ratios as seen in normal liver and heart mitochondria.

Significance to Biomedical Research and the Program of the Institute: Cancer cells differ from most normal cells in utilizing anywhere from 5 to 15 times as much glucose from the blood per unit weight per unit time as normal cells. They convert glucose, a neutral molecule, into lactic acid, which ionizes to yield H^+ and the lactate ion, which diffuses into the blood. These characteristic changes are apparently the consequences of the genetic peculiarities of tumors that result in expression of fetal isozyme patterns. This process of aerobic glycolysis of glucose, which in principle renders cancer cells parasitic on normal cells in the body, thus robs adjacent normal cells as well as the liver of significant amounts of energy. Study of the membrane transport mechanisms responsible for carrying glucose into the cancer cell and lactic acid out, together with the H^+ generated by glycolysis and respiration, may help to unravel the nature of the metabolic regulatory mechanisms that undergo epigenetic change during carcinogenesis. Moreover, the profound changes in the distribution of H^+ ions between the mitochondrial, cytosolic, and extracellular compartments of tumor cells also shed light on these regulatory mechanisms and may lead to means of specific control of cancer cell growth or of the systemic effects of cancer on the host.

This set of projects is aimed toward the solution of one of the problems facing the National Cancer Institute, namely, the definition of the enzymatic and membrane transport processes that are altered by the process of carcinogenesis, whether spontaneous or induced by exogenous chemical agents. It may also have a long-range application to chemotherapeutic problems.

Proposed Course: It is now proposed to examine the stoichiometry of respiration-coupled ejection of H^+ ions from mitochondria isolated from a number of tumor cell lines to determine the generality of the fundamental stoichiometry of energy conservation in the form of ATP. It is a second objective to determine the effect of changes in acid-base balance on a regulation of tumor cell activities and to devise new affinity inhibitors for the lactic acid transport system.

It is also proposed to extend our studies of Ca^{2+} transport and release by the mitochondria of tumor cells, which are very relevant to the regulation of the cell cycle, as well as the tendency of some cancers to metastasize to bony tissue and of others to metastasize to soft tissue. Determination of the steady-state concentration of Ca^{2+} within tumor is to be undertaken to determine which of the many Ca^{2+} dependent activities are most influenced by the cytosolic Ca^{2+} concentration.

We also propose to search for other transport systems of the plasma membrane of tumor cells, particularly those involving efflux of tumor-generated compounds capable of exerting systemic effects on neighboring normal cells. In our future work we also intend to utilize virus-transformed cells more extensively in order to demonstrate more conclusively that the effects observed are due to the malignant change.

Date Contract Initiated: June 28, 1974

Current Annual Level: $158,441

MANITOBA, UNIVERSITY OF (NO1-CP-43250)

Title: Isolation, Purification, and Characterization of Human Prolactin

Contractor's Project Director: Dr. Henry G. Friesen

Project Officers (NCI): Dr. Marie Green
Dr. Andrew C. Peacock

Objectives: (1) The purification of human prolactin from pituitary glands and from amniotic fluid, and (2) the chemical characterization of human prolactin including the elucidation of the complete amino acid sequence of human prolactin.

Major Findings: Extraction and purification of pituitary prolactin has been continued by the contractor using methods which were developed at the University of Manitoba. The procedures have been applied to the purification of prolactin both from acetone-dried human pituitary powder and from frozen pituitary glands. The latter are a superior source for purification purposes because the yields of prolactin are considerably greater than those obtained from acetone-dried powder. The methods employed have proven to be reasonably satisfactory and have yielded a product with a potency of 25-30 IU/mg, which is equal to that of sheep prolactin standards.

Amino acid sequence studies are being continued in collaboration with Dr. Niall of the Florey Institute in Australia. To date, approximately 90% of the total amino acid sequence of human pituitary prolactin has been elucidated.

This year 1000 frozen pituitary glands have been obtained from the Medical Research Council of Canada for extraction of pituitary prolactin. In addition, 10,000 acetone-dried human pituitaries have been processed for human growth hormone according to the Raben procedure. The human prolactin side fraction (4.88 g at 0.32% h.PRL) contained 15.6 mg. of h.PRL.

Amniotic fluid is being collected in a number of centers for extraction and purification of prolactin. The procedures for purification of amniotic fluid prolactin include standard precipitation procedures, affinity chromatography, and isoelectric focusing on a preparative scale. Some new techniques pioneered by Dr. Chrambach of NIH are being tried which are likely to be suitable for improving purity (with reasonable yield) of human prolactin isolated from amniotic fluid. Buffer electrofocusing was tried as a substitute for commercially available ampholines. It produced a reasonable pH gradient and separation of proteins and shows considerable promise. Steady-state stacking was also tried as a method to concentrate samples off a number of gel segments. Instead of relying on diffusion to elute material out of polyacrylamide gels, the required material can be electrophoretically driven into a "stack" and this "stack" drifted off into a small volume cup. This technique is useful for concentrating material from a volume of about 100 ml to 1 ml.

Six mg of h.PRL were used to immunize 6 rabbits, of which 2 produced antisera of reasonable titre and 1 produced excellent titre. Attempts to immunize

larger animals (such as sheep, goats, or donkeys) will be made in the near future.

Dr. Niall and his colleagues are continuing to perform amino acid sequence analysis of prolactin. With the availability of new micro procedures, the total amount of human prolactin which is necessary to complete the amino acid sequence should be considerably lower than when these studies were begun. Dr. Niall reported on the sequence of 80-90% of the molecule at the Vth International Congress of Endocrinology in Hamburg.

During the course of this contract, plasma membrane receptors for prolactin were identified in a number of tissues. The identification and characterization of rabbit mammary cell receptors for prolactin have been utilized for the development of the radioreceptor assay for prolactin and other lactogenic hormones. This assay proved to be very helpful in monitoring prolactin during the purification procedures. It is proving very useful for structure-function studies in prolactin and other lactogens. The particulate preparation containing the prolactin receptor has been solubilized and the receptor purified. The availability of a purified solubilized prolactin receptor should lead to more reproducible assay results. In addition, it should facilitate studies on structure-function relationships. Antibodies to the purified prolactin receptor have been developed which reveal that there is immunochemical homology among prolactin receptors of different tissues. Antibodies generated against prolactin receptors from rabbit mammary tissues inhibit the action of prolactin. The antibodies cross react with prolactin receptors in human tissues. The detection and quantitation of prolactin receptors in benign and malignant human mammary tumors is continuing.

Significance to Biomedical Research and the Program of the Institute: The purification and characterization of human prolactin has significant implications to the overall problem of carcinogenesis. Prolactin has been implicated in the development of two of the most common types of cancer: mammary and prostatic carcinomas. The data linking prolactin to these two forms of cancer is extensive in experimental animals; in human subjects, the evidence is much less clear. One of the major reasons for the obscure relationship in man is based on the fact that human prolactin was not identified as a separate human pituitary hormone until 1971. At the moment, more extensive research on human prolactin is impossible because of the very limited amounts of hormone available. One of the major aims of this contract is to continue exploration of methods which will improve purification procedures currently employed and, thereby, to increase the available supply of this hormone. This will allow further research on human prolactin and its role in carcinogenesis in man. To assist other investigators in their studies on the role of prolactin in health and disease, purified prolactin and antisera to human prolactin have been made available to investigators worldwide.

Proposed Course: Continue the purification and characterization of human prolactin from human pituitaries and amniotic fluid.

Date Contract Initiated: June 30, 1972

Current Annual Level: $65,614

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-43265)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald N. Wogan
Dr. George H. Büchi
Dr. Arnold L. Demain

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: To investigate the biochemical, toxic, and carcinogenic effects of aflatoxins, their metabolites, and analogs; to determine the metabolic fate of aflatoxins in various species; to synthesize aflatoxin metabolites; and to isolate, characterize, and investigate the toxicity and carcinogenicity of other mycotoxins produced by molds isolated from foods or food raw material.

Major Findings: Toxicity and carcinogenicity evaluations of aflatoxins M_1, P_1, and Q_1 are in progress. Attempts are being made to separate and identify metabolites of aflatoxin B_1 in urine and DNA-aflatoxin B_1 adducts using high-pressure liquid chromatography. Isolates of toxin-producing fungi previously collected from foods in Thailand are being cultured on various media to provide material from which new mycotoxins can be isolated. During the current year, the following compounds have been isolated and structurally identified: nortryptoquivaline, nortryptoquivalone, tryptoquivaline, deoxynortryptoquivalone, deoxynortryptoquivaline, deoxytryptoquivaline, cytochalasin E, kotanin, desmethylkotanin, 7-hydroxy-4-methoxy-5-methylcoumarin from Aspergillus clavatus; kojic acid, cyclopiazonic acid, sterigmatocystin and O-methylsterigmatocystin from Aspergillus flavus; cyclopiazonic acid from Aspergillus tamari and sterigmatocystin, ergosterol and averufin from Aspergillus versicolor. Work is continuing on these and other additional mold cultures.

Significance to Biomedical Research and the Program of the Institute: Evidence is accumulating that chemical products arising from microbial spoilage (particularly involving mold-damage) of foods or food raw materials can appear in the human food supply and may be associated with the etiology of liver cancer in several areas of Asia and Africa. The scope of the problem in terms of numbers of compounds and/or molds that may be involved has not yet been accurately defined but is potentially great. This project, which is related to other NCI contracts on naturally-occurring carcinogens, is designed to produce relevant information on these questions.

Proposed Course: Experiments will continue along the lines suggested by the objectives outlined above. Studies on the comparative metabolism of aflatoxins may help to delineate the mechanisms involved in rather wide species differences in response to these compounds. Similarly, studies on the biochemical effects, toxicity, and carcinogenicity of analogs and metabolites may provide evidence on active forms of the agent and on sites of action. Identification of new mycotoxins from other molds isolated from food samples will provide further definition of the scope and character of the potential hazards to man represented by these toxins.

Date Contract Initiated: June 25, 1962

Current Annual Level: $ 240,499

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33238)

Title: Interactions Between Diet and Chemical Carcinogenesis: A Bioassay System

Contractor's Project Director: Dr. Adrianne E. Rogers

Project Officer (NCI): Dr. Lionel Poirier

Objectives: (1) To develop in rats a bioassay model for carcinogens using lipotrope deficiency which reproduces nutritional conditions and effects under which people may be exposed. (2) To define the specific nutrients responsible for dietary effects on carcinogenesis which are observed.

Major Findings: A semisynthetic diet, which provides adequate levels of nutrients for growth of rats but is marginally deficient in the lipotropes choline, methionine, and folic acid and high in fat (1) enhances induction of liver tumors by 5 of 6 carcinogens tested, (2) enhances induction of colon tumors by DMH, (3) does not affect induction of tumors of the stomach or urinary bladder, (4) decreases induction of mammary tumors by DMBA and under some conditions by AAF, (5) alters urinary metabolites of AAF in a manner consistent with changed AAF carcinogenic activity. Supplementation of the deficient diet with lipotropes (choline, methionine, and folic acid) or with limiting amino acids, including methionine, decreases induction of hepatocellular carcinoma by DENA almost to the level found in rats fed the control, fully adequate semisynthetic diet; addition of fat to the control diet does not increase hepatocellular carcinoma induction. Supplementation of the deficient diet with lipotropes or amino acids does not decrease induction of colon tumors by DMH; addition of fat to the control diet enhances tumor induction. Addition of fat to the control diet decreases mammary tumor induction of AAF in female rats. Studies of the effect of diet supplementation on AFB_1 carcinogenesis are in progress.

Significance to Biomedical Research and the Program of the Institute: Dietary effects on chemical carcinogenesis can be demonstrated in experimental animals and are strongly indicated by epidemiologic studies in humans. Development of bioassay systems in animals utilizing known dietary effects will (1) permit definition of the dietary components which may retard or enhance chemical carcinogenesis, (2) provide models in which study of metabolism of carcinogens and mechanisms of carcinogenesis can be made and related to differences in effective carcinogenesis, and (3) provide a sensitive system for testing compounds which give equivocal results in normal rats.

Proposed Course: (1) Completion of present experiments comparing tumor induction in normal or lipotrope-deficient rats by carcinogens of different

chemical classes and affecting different target organs, and (2) definition of the specific dietary component(s) responsible for the enhancement of carcinogenesis by the lipotrope-deficient diet.

Date Contract Initiated: February 1, 1972

Current Annual Level: $147,028

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33315)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractor's Project Directors: Dr. Steven R. Tannenbaum
Dr. Michael C. Archer

Project Officers (NCI): Dr. Elizabeth Weisburger
Dr. Larry Keefer

Objectives: The current objectives of this contract are: 1) to investigate how nitrate is metabolized in man, particularly how it is converted to nitrite; 2) to investigate formation of N-nitroso compounds by deliberate nitrosation of food material under simulated gastric conditions; 3) to investigate formation of N-nitroso compounds from primary amines in saliva and gastric contents; 4) to apply the findings of objectives 1-3 to the etiology of gastric cancer in Southern Colombia.

Major Findings: A microprocedure for analysis of nitrate has been developed. Normal diet and possibly endogenous sources were shown to contribute significantly to nitrate levels in human urine and saliva and hence to salivary nitrite. Gastric contents of 78 individuals from a region with a high incidence of gastric cancer in Southern Colombia have been analyzed. The highest values of nitrite were over 200ppm and it appears that high stomach pH is essential for high nitrite. Concurrent high values of thiocyanate were also found, but the major ion in the gastric contents was chloride.

A number of different grains and vegetables from Southern Colombia and similar materials from North America have been nitrosated under simulated gastric conditions. All of the samples formed a variety of volatile N-nitroso compounds. Several of the compounds occurred in a number of different samples. The major compounds are currently being identified. A procedure has been developed for analysis of nitrosoproline in raw bacon. Results indicate that preformed nitrosoproline is not a major source of nitrosopyrrolidine.

Several primary amines have been shown to form stable N-nitroso compounds when added to saliva. The nitrosation reaction occurs more readily in whole saliva than salivary supernatant or resuspended cellular material. Thiocyanate has been shown to catalyze formation of these N-nitroso compounds. Their structures are currently under investigation, but they appear not to be primary nitrosamines.

Rate enhancements of from 12- to 40-fold occurred when dihexylamine was nitrosated at pH 3.5 in the presence of bacteria and yeast cells at a concentration of 12 mg/ml. Catalytic rates were similar in the presence of either boiled or unheated cells. The magnitude of the rate enhancement for nitrosation of other amines depends on the alkyl chain length. A nonenzymatic mechanism involving hydrophobic interactions of the precursor amines and cellular constituents has been proposed.

A quantitative method has been used to illustrate that evaluation of the potential dangers of toxic substances using concentration levels alone may lead to erroneous conclusions.

Significance to Biomedical Research and the Program of the Institute: N-nitroso compounds may be the cause of certain types of human cancer. If conditions could be found in the environment which allow correlation of N-nitroso compound formation with a specific form of cancer, the knowledge could be applied to prevention of the disease. This project will determine the total extent to which N-nitroso compounds may be present in various environments including a cancer-rich region of Colombia. Conditions which enhance or retard formation of N-nitroso compounds in foods, beverages, human saliva, and the stomach will be investigated.

Proposed Course: A major emphasis in this program will continue to be N-nitroso compound formation and occurrence in a cancer-rich region of Colombia. Research will continue on conversion of nitrate to nitrite in man, nitrosation of food materials under gastric conditions, and formation of N-nitroso compounds in saliva and gastric contents.

Date Contract Initiated: June 11, 1970

Current Annual Level: $161,996

MICROBIOLOGICAL ASSOCIATES (NO1-CP-55605)

Title: Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Richard E. Kouri

Project Officer (NCI): Dr. Paul Okano

Objectives: To define the relationship between levels of the enzyme systems, aryl hydrocarbon hydroxylase (AHH), and cancer susceptibility in the human population. Emphasis was directed towards standardization of the AHH assay, especially as it applies to samples obtained from Creighton University, and determination of the relationship between levels of AHH and genetically-regulated expression of cancer using "cancer-free" and "cancer-prone" families.

Major Findings: The assay of a total of sixty normal individuals, for a total of 580 assay points, has shown that at least several factors influence the levels of AHH activity in human lymphocytes. The initial concentration of lymphocytes seeded determines the kinetics of mitogen activation and, by definition, the amount of AHH activity. Since the number of lymphocytes per ml whole blood can vary by 100% both between people and within one person when bled at different times, the culturing of lymphocytes on a per ml whole blood basis, rather than on a per cell basis, results in an assay where not only the peak AHH levels are affected, but also the time of occurrence of these peaks are different. The inducer of AHH activity (e.g., 3-methylcholanthrene) must be on the cells long enough to insure maximal induction. Under culture conditions used in this laboratory, at least a 48-hour induction period is required. The kind or lot of serum supplement must be controlled. This parameter may not be completely controllable because some inherent individual-to-individual variations in the response to different lots of fetal calf serum are observed. In this laboratory, the use of pooled human AB^+ serum is the major single factor in improving the reproducibility of mitogen activation step. Further, during the mitogen-activation steps, the assay for AHH activity must be done at more than one time. That is, depending on culture condition, peak AHH activity can be observed at 72, 96, or 120 hours of mitogen activation. If the initial cell concentration is about 1.0 x 10^6 cells/ml, current culture conditions yield peak AHH levels at either 72 or 96 hours of mitogen activation. The parameters on which to base these observed AHH activities are either total DNA or the level of NADH-dependent cytochrome c reductase activity. Assay of 10 individuals a total of five different times according to the above conditions yielded an average within assay variation of individuals of ca. 0.10 (C.V.), while the between assay variation of these same individuals was ca. 0.20 (C.V.).

The parameter, AHH/cytochrome c reductase, was the most reproducible way to present the data. Reproducible differences in the induced AHH levels were observed between certain individuals, and these differences could not be accounted for by differences in degree of mitogen activation. That is, the rates of incorporation of ^{3}H-thymidine into acid precipitable material (DNA sysnthesis) was similar in these individuals.

The radiometric assay for conversion of ^{3}H-benzo(a)pyrene to water-soluble forms has also been evaluated and data suggest that the assay may be at least as reproducible as the fluorometric assay. However, the differences between individuals seem to be quite small. Thus, this assay may be more reproducible but less discriminating than the fluorometric method.

Significance to Biomedical Research and the Program of the Institute: The determination of the biochemical basis of at least certain forms of cancer in man is of obvious significance to biomedical research and the program of the Institute. AHH levels may not only be a major determinant in man's ultimate susceptibility to chemically-induced cancers, but it might still augur the possibility of new approaches to the control of cancer. However, before this parameter can be evaluated realistically, a more reproducible assay than the one presently available must be developed.

Proposed Course: Contract expired September, 1976.

Date Contract Initiated: September 9, 1974

Current Annual Level: $73,805

MISSISSIPPI, UNIVERSITY OF (NO1-CP-43347)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. John K. Baker

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) Develop analytical methods for N-nitroso amino acids, (2) develop analytical methods for other N-nitroso compounds using high pressure liquid column chromatography, and (3) assist NCI contractors at the Thermo Electron Corporation in the development of the thermal energy analyzer.

Major Findings: The operating characteristics of the thermal energy analyzer detector have been extensively investigated in its use as a detector for high pressure liquid chromatography. It was found that polar nitroso compounds having very low vapor pressures had response characteristics that were markedly different from those of the volatile nitrosamines. It was found that the optimum operation temperature for the nitroso amino acids was 350°, while the optimum temperature for the volatile nitrosamines was higher.

Analytical methods for the nitroso amino acids in meat samples were developed. Analysis of samples submitted by the World Health Organization containing several nitroso amino acids were also analyzed as part of an international program for the comparison of different analytical methods.

Significance to Biomedical Research and the Program of the Institute: At the present time, analytical methods are available only for a very limited number of carcinogenic N-nitroso compounds in food products or the environment. It is speculated that the N-nitrosopyrrolidine and other volatile nitrosamines that have been observed in cooked meat samples may be formed during the thermal decomposition of the corresponding nitroso amino acid. In order to estimate the potential production of the carcinogenic nitrosamines in the cooked food, it would be desirable to be able to determine the nitroso amino acid content of the uncooked food product.

A second function of the project was to investigate the basic operating characteristics of the TEA-HPLC system and provide this information to other NCI contractors who will be receiving these units in the near future.

Proposed Course: Complete the development of methods for the detection of N-nitroso amino acids in food products; then apply these methods on a variety of commercial food products. Efforts will then be made to detect and identify other N-nitroso compounds in these products.

Date Contract Initiated: May 1, 1974

Current Annual Level: $32,067

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CP-33279)

Title: Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Claudio Basilico

Project Officer (NCI): Dr. John P. Bader

Objectives: (1) Isolation and characterization of temperature sensitive (ts) mutants of somatic animal cells; and (2) use of these mutants to study mutation mechanisms in somatic animal cells, and the effect of specific biochemical lesions on the regulation of cell growth and division.

Major Findings: Over 90 ts mutants of the hamster cell line BHK 21 are isolated. These mutants exhibit low reversion and low leakiness. Their ts growth is due to a variety of defects as shown by genetic and biochemical analysis. All mutations tested are recessive. Complementation tests have assigned the mutants tested so far to 25 complementation groups, with a few groups containing more than one mutant. The ts 422E mutant cannot grow at 39° because of a defect in the processing of ribosomal RNA precursors, leading to a block in the production of 28S ribosomal RNA. This defect is probably due to the presence of a ts ribosomal protein. The ts 422E mutation can be directly complemented in intra- and inter-specific hybrids. Mutant ts AF8 becomes arrested at 39° in the G1 phase of the mitotic cycle. Synchronization - shift-up experiments place the cell cycle block of this mutant in early G1 between the blocks induced by serum starvation and that induced by isoleucine deprivation. Transformation by polyoma virus alters the phenotypic expression of this mutation. Three other mutants, which at 39° become arrested in G1, have been studied. These mutants complement with ts AF8, and the cell cycle block in G1 appears to be distal to the AF8 block. One of these mutants may be directly affected in the initiation of DNA synthesis. Other cell cycle mutants, including one which at the non-permissive temperature is blocked in G2, have been isolated and are being studied. Recently the isolation of ts mutants from a line of Fisher rat cells, F2408, has also been undertaken.

Significance to Biomedical Research and the Program of the Institute: Cancer cells have altered regulation of proliferation and differentiation. Conditional lethal ts mutations have proven to be extremely useful for a variety of studies with microorganisms, and their usefulness to further our understanding of somatic animal cells and their regulatory mechanisms is becoming evident.

Such temperature sensitive mutants, in particular those affected in macromolecular synthesis, cell cycle regulation, and possibly membrane formation, are extremely valuable in studies of carcinogenesis. They should provide an understanding of the cell regulatory mechanisms and of their breakdown during neoplastic transformation, which is basic to our understanding of cancer.

Proposed Course: Future work is aimed at the following objectives: (a) the isolation and study of additional ts mutants from the BHK hamster line, particularly cell cycle mutants which at high temperature are defective in functions required by G1 or G2 progression. In addition, ts mutants will be isolated from a new cell line of rat fibroblasts, F2408; (b) the further characterization of the mutants already obtained from the genetic and biochemical point of view; (c) improvements of the mutagenesis and selection techniques to make them both more effective and specific for given classes of mutants; (d) the use of some of the mutants for specific experiments, particularly the study of the cell functions required for cell cycle progression, the investigation of what determines cell transition from a growing to a resting state and the effect of viral transformation on this process.

Date Contract Initiated: June 26, 1971

Current Annual Level: $162,967

OHIO STATE UNIVERSITY (NO1-CP-43248)

Title: Preparation of Polymers Containing Carcinogenic Moieties

Contractor's Project Director: Dr. Melvin S. Newman

Project Officer (NCI): Dr. Shen K. Yang

Objective: To prepare polymers in which the benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene ring systems are attached to a polymer chain by a non-biodegradable linkage. Similar polymers in which non-carcinogenic ring systems are attached are also desired as control polymers.

Major Findings: The following polymers were prepared and are availabe to interested researchers on demand: (Requests should be addressed to the contractor's project director.) poly-p-(1-benzo[a]pyrenylmethoxymethyl) styrene, poly-p-(6-benzo[a]pyrenylmethoxymethyl)styrene, poly-p-(7,12-dimethyl-10-benz[a]anthracenylmethoxymethyl)styrene, poly-1-vinylbenzo[a]pyrene, and poly-1-vinylpyrene.

In addition, quantities of 10-methylbenzo[a]pyrene and 7,10-dimethyl-benzo[a]pyrene are available to test current hypotheses concerning metabolism of benzo[a]pyrene leading to the ultimate carcinogen. Unpublished results by James A. and Elizabeth C. Miller have established that 10-methylbenzo[a]pyrene is inactive as a carcinogen.

Significance to Biomedical Research and the Program of the Institute: The fact that 10-methylbenzo[a]pyrene is inactive, and presumably 7,10-dimethylbenzo[a]pyrene will also be inactive is of timely interest in connection with the studies involving the 7,8-diol-9,10-epoxide derivative of BP. These compounds offer substrates which can be used to test current theories as to metabolic activation of BP.

The testing of the polymers available is of importance, for if the vinylbenzo[a]pyrene polymer proved to be carcinogenic it would mean that cancer could be caused by an agent which does not enter the cells involved.

Proposed Course: The objectives of this project have been completed this fiscal year.

Date Contract Initiated: January 11, 1974

Current Annual Level: $28,160

ONTARIO CANCER INSTITUTE (NO1-CP-43331)

Title: Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Louis Siminovitch

Project Officer (NCI): Dr. John P. Bader

Objectives: (1) Isolation of cell lines carrying mutations affecting key cell functions such as protein synthesis, or key cell structures such as the plasma membrane. (2) Biochemical and physiological characterization of the mutant lines. (3) Examination of the properties of hybrid cell lines obtained by fusion of cells of one mutant line with other normal or mutant lines, and the use of chromosome transfer techniques for complementation analysis, possible mapping, and for studies of chromosome behaviour and gene control. (4) Extension of the techniques, as they are developed, to a variety of cell types chosen not only on the basis of their suitability for studies of the regulation of cell multiplication and differentiation but also for other purposes, such as the detection of environmental carcinogens and mutagens.

Major Findings: Work has continued on the development of methods to select mutants of Chinese hamster ovary (CHO) and other cells, and on their characterization by both genetic and biochemical criteria. The conditional lethal temperature-sensitive (ts) tRNA synthetase mutants of CHO cells have been used as a means of shutting down protein synthesis at nonpermissive temperature in a manner distinct from the effect of cycloheximide. This will allow the development of models for control processes and cell killing. Evidence has been obtained that patients being treated with chemotherapeutic agents show the same pleiotropic phenotype as CHO colchicine resistant cells, since they show resistance to adriamycin, dainomycin, and puromycin. Complementation tests on CHO mutants resistant to 5 lectins have shown that all but one

of the mutants (Ric^{RII}) behave recessively in this test, and that 8 complementation groups can be identified in this way. Three classes of mutants resistant to methotrexate have been isolated in single-step selections in CHO cells. Mtx^{RI} cells contain an altered dihydrofolate reductase, as determined by the increased resistance of the enzyme in vitro, Mtx^{RII} cells owe their resistance to altered transport of the drug, and Mtx^{RIII} cells, which are derived from Mtx^{RI} as a second mutation, contain 10-fold increased activity of resistant enzyme. Mtx^{RI} and Mtx^{RIII} behave codominantly, and Mtx^{RII} recessively, in somatic cell hybrids. CHO mutants resistant to emetine, tylocebrine and cryptopleurine are identical and contain a modification in the 40S ribosomal subunit. The mutants behave phenotypically as recessives.

Studies of chromosome karyotype in CHO cells indicate that, although this karyotype is quite abnormal, there is very little variation from cell to cell and during long periods in culture of quasi-diploid cells. Such variation is much more extensive in quasi-tetraploid cells. Chromosome segregation is increased markedly in going from quasi-tetraploid to quasi-hexaploid cells and on treatment with a variety of mutagens. Selection for thioguanine-resistant TG^R cells from a hybrid of a TG^R and a wild-type cell ($hprt^+/hprt^-$) results in loss of the X, or a piece of the X chromosome. Thus, segregation in this case can be equated with chromosomal loss. Chromosomes from cells carrying the dominant markers Mtx resistant (Mtx^{RIII}), Ouabain resistance (Oua^R) and α-amanitin resistance (Amal) have been transferred to recipient cells and selected in the presence of the relevant drug. A rough chromosome fractionation places the Mtx^R marker in the middle-size class and Oua^R marker in the large-size class of chromosomes. When Mtx^{RIII} is used as the donor both the structural enzyme and the increased level are transferred, indicating either that the two genes are very closely linked or that the second-type mutation involves gene duplication. Stabilization of the marker in the absence of selection is observed in some clones. Cells from Fanconi patients show increased sensitivity to mitomycin C and decreased sensitivity to mutagenesis. Thus they have properties similar to the recA mutants in bacteria. Studies on mutation to ouabain resistance in human diploid fibroblasts show that mutagens such as EMS, MNNG, and UV light are all effective in increasing the frequency of Oua^R colonies obtained, and factors such as cell density at selection, ouabain concentration, and the interaction between the dose of mutagen and the length of expression time also affect the yield of mutants. Mutants of various degrees of resistance to α-amanitin have been isolated from rat myoblast cells, and such mutants contain RNA polymerase II which is both resistant to the drug and shows deficient binding. The levels of the latter parameters relate to the degree of resistance of the cells to the cytotoxicity of α-amanitin. Treatment of resistant rat myoblast cells with α-amanitin results in inactivation of the sensitive enzyme and a concomitant increase in activity of the resistant enzyme. Binding studies indicate that the addition of the drug to the enzyme probably results in some degradation of the active subunit.

Significance to Biomedical Research and the Program of the Institute: Cancer may be regarded as a disease in which cells are genetically altered such that their regulation of cellular multiplication and differentiation is defective. Temperature-sensitive mutations affecting key functions involved in cell

growth and, in particular, DNA replication and mitosis are of specific interest in respect to obtaining an understanding of the breakdown of regulation during carcinogenesis. Suitably characterized cell lines are useful not only for studies of cellular regulatory processes but for other purposes as well. One example is the use of cell-culture systems for the detection of environmental carcinogens or mutagens. Our extensive list of markers will be of considerable use in this respect. In addition, studies of the genetic basis of drug resistance are of special interest in respect to chemotherapy. For example, our finding that mutation to resistance of colchicine and to Con A engenders collateral changes in sensitivity to other drugs must be considered in contemplation of multiple drug trials, and our findings on methotrexate resistance are of particular interest because of the extensive use of this drug in chemotherapy. The several dominant markers isolated in our laboratory will be of considerable use in studies on gene transfer which, in turn, may allow analysis of the genetic basis of the neoplastic phenotype.

Proposed Course: To continue with the studies as outlined above.

Date Contract Initiated: January 1, 1972

Current Annual Level: $184,000

OREGON STATE UNIVERSITY (NO1-CP-43348)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. Richard A. Scanlan

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) To analyze foodstuffs or environmental samples for individual N-nitroso compounds and their precursors using established methods, (2) to examine new analytical techniques which may become available, and (3) to conduct other research aimed at understanding the formation, identification, quantitation and destruction of N-nitroso compounds in the human environment.

Major Findings: 3-Hydroxy-N-nitrosopyrrolidine was extracted from fried bacon and fried-out fat with water:methanol (3:2). After elimination of residual nitrite with ammonium sulfamate, the nitrosamine was removed from the water:methanol by continuous extraction with dichloromethane. Identification was accomplished by conversion of 3-hydroxy-N-nitrosopyrrolidine to the trimethylsilyl derivative followed by separation on a capillary column and characterization by gas chromatography mass spectrometry.

Quantitation and recovery studies were conducted without derivatization. Concentrated dichloromethane extracts were analyzed using a gas chromatographic thermal energy analyzer system. Recovery of 3-hydroxynitrosopyrrolidine from fried bacon and fried-out fat was 62 and 63%, respectively. Five commercial samples of fried bacon contained 0.4-3.9 ppb, while the fried-out fat

contained 0.3-2.2 ppb 3-hydroxy-N-nitrosopyrrolidine. It should be pointed out that these analyses could not have been accomplished without the thermal energy analyzer which is on loan from the NCI.

Significance to Biomedical Research and the Program of the Institute: 3-Hydroxy-N-nitrosopyrrolidine was isolated and identified in fried bacon and in fried-out fat for the first time. Based on a limited number of determinations, the nitrosamine appears to be present in bacon in the lower parts per billion level. At present, the carcinogenic potential of 3-hydroxy-N-nitrosopyrrolidine is not known. The results add to our knowledge concerning the occurrence of N-nitroso compounds in the environment.

Proposed Course: To continue analyzing samples of interest and to elucidate the mechanisms of nitrosamine formation in foods. In addition, methods for the analysis of nonvolative nitrosamines will be developed. The TEA on loan from the NCI will be used in these investigations.

Date Contract Initiated: May 2, 1974

Current Annual Level: $40,518

POLYSCIENCES, INC. (NO1-CP-23245)

Title: Optimizing Electrophoretic Separation of Proteins and Nucleic Acids with New Hydrogels

Contractor's Project Director: Dr. B. David Halpern

Project Officer (NCI): Dr. Andrew C. Peacock

Objective: To study the optimization of electrophoretic separation of proteins and nucleic acids in new hydrogel media through the modification of the following gel properties: (1) Pore Size: Develop gels with large enough pore size yet maintaining optimal sieving properties to permit the study of large biological macromolecules such as r-RNA, DNA, and ribosomes. (2) Associative Effects: Utilize the associative effects (such as hydrogen bonding, electrostatic effects, etc.) between the biological macromolecule and the polymer backbone of the gel medium and such other effects caused by solvent, external additives, etc., for optimal resolution. (3) Elutibility: Design and develop gels which are easily resolubilized or otherwise suited for simple recovery of the biological sample after electrophoresis without denaturization of the protein.

Major Findings: Part of the work done previously with gels obtained from polyethylene glycol (PEG) using proteins and 16S + 23S r-RNA was repeated, using more highly purified monomers. Polyethylene glycol 14,000 diacrylate was added to the series. These diacrylates gave gels either when polymerized alone or with the incorporation of acrylamide (AA). The straight PEGDA gels generally gave gels of larger pore size than AA/MBA gels of equivalent total

concentration (T), but when used as crosslinkers with AA the pore size was smaller. The advantage observed in the larger pore size showing increased relative mobilities in these gels was not commensurate with the additional length of the crosslinker. A further careful study of literature revealed that the various high mol. wt. PEGS were complex mixtures of linear and non-linear components in a wide range of mol. wts. Therefore, it was decided to use diethylene glycol diacrylate and triethylene glycol diacrylate which could be obtained in 97-98% purity for the preparation of these hydrogels. These gels showed definitely larger pore size as compared to AA/MBA gels of same concentration and molar ratio. But this advantage did not hold when larger mol. wt. PEGDAs were used, as mobilities of the model compounds were not much affected.

This observation led to the belief that other secondary factors such as hydrogen bonding may be important in these gels. To check the validity of the pore size concept, a series of gels was designed in which hydrogen bonding could be kept to the minimum and the only factor under study would be the pore size of the gel as dependent on the length of the crosslinker. For this purpose, a series of methylenebisacrylamide crosslinkers were synthesized with values of $(CH_2)_n$ varying from $n=2$ to $n=8$. These were used as crosslinkers, replacing MBA in standard AA/MBA gels, in the same molar ratio. Even if the length of the crosslinker was systematically increased, no systematic increase in pore size was noticeable.

In order to further check the idea of hydrogen bonding, a preliminary study was carried out with gels made from N-methylolacrylamide and N,N'-dihydroxyethylenebisacrylamide which together provide three -OH groups over the AA/MBA gels. It was observed that this gel, while it should provide a relatively larger pore size than AA/MBA gel because of the greater length of the crosslinker than MBA, actually gave a tighter gel in which proteins moved much slower than in the standard AA/MBA gel. The advantage of such gels might be that they will have reasonably good mechanical strength and handling quality even at low concentration, such as 2 to 2.5%, without having to use agarose. At that concentration, it may be possible to analyze and resolve higher mol. wt. biomolecules than what is possible with AA/MBA gels. Work on this gel and on similarly constituted other gels is in progress. These gels are also easily reversible, which will aid in the isolation of the separated bands without loss or damage.

Significance to Biomedical Research and the Program of the Institute: This work should lead to ways and means of making gels of good mechanical strength at low cost which will permit separation and isolation of large biomolecules such as ribosomes, ribosomal RNA, and DNAs according to their mol. wts. and conformational characteristics, thereby aiding in identification of their normal or carcinogenic source.

Proposed Course: Completion of the study of gels made with AA and the polymethylenebisacrylamides from $n=1$ to $n=8$. Preparation of gels from various monomers and crosslinker offering varying degree of hydrogen bonding. Preparation and study of gels made from polyfunctional branched monomers so as to give gels of good mechanical strength at lower concentrations.

Date Contract Initiated: May 17, 1972

Current Annual Level: $79,611

ROCHESTER, UNIVERSITY OF (NO1-CP-45611)

Title: Mammalian Cell Transport Systems

Contractor's Project Director: Dr. John Donald Hare

Project Officer: Dr. Tsuyoshi Kakefuda

Objectives: 1) To develop methods with which to identify and isolate the proteins associated with nucleoside and the several amino acid transport systems in mammalian cells. A principal method of approach is the development of photoactive substrate analogues with which to label the putative transport proteins and to follow their isolation. 2) To determine the structure and function of these transport proteins in normal cells and how they might be altered either in structure or function following neoplastic transformation.

Major Findings: A number of photoactivable substrate analogues have been synthesized and screened as potential probes to identify specific transport associated proteins. One analogue of adenosine, N6-(Nitrophenylazido)-adenosine (ANPA) has been shown to inhibit nucleoside transport reversibly in the dark and to inhibit irreversibly after photolysis, a phenomenon associated with covalent binding to membrane proteins. A second analogue, 8-azido-adenosine, has been shown to be a good substrate for the nucleoside transport system but fails to photoinactivate transport. Several photosensitive analogues of phenylalanine and tyrosine have been found to act as substrates for the L transport system in tissue cultured cells. Azido-phenylalanine photoinactivates phenylalanine transport approximately 25-50% but inactivates nucleoside transport 90%. Azido-tyrosine fails to inactivate nucleoside transport, pointing to a feature of its ring structure which alters the non-specificity toward the site in the nucleoside transport system sensitive to the hydrophobic azido-phenylalanine ring structure. It is suggested that hydrophobic substituents on the 6 position of several adenosine analogues including ANPA interact with a hydrophobic region of the nucleoside transport system which is also recognized by the azido-phenylalanine. Thus, special features of the various transport systems must be considered in designing probes with the proper selectivity to optimize binding and subsequent photactivated labeling.

A second finding of note has been the difference in lipid content between normal hamster embryo cells and a line of polyoma transformed hamster tumor cells. Phosphotidylcholine (PC) and phosphotidylethanolamine (PE) were decreased and phosphotidylthreonine (PT) was increased in the transformed cells. The PE of transformed cells also reacted to a greater extent with FDNB and TNBS while PE and PT failed to react with TNBS in either cell type. The tumor cells were also found to have a higher cholesterol ester to cholesterol ratio, pointing to further variation in the lipid composition of the tumor

cell membrane system. Whether these changes are related to transformation in a direct or indirect way is under investigation.

Significance to Biomedical Research and the Program of the Institute: The structure and function of the cell membrane has come to be recognized as an important intermediate in the mechanism by which a cell in the tissue and organs of higher organisms, including the human, is regulated both in cell function and cell division. The detailed molecular mechanisms by which nutrient substrates are taken up by the cell has not yet been fully elucidated. Furthermore, the role played by modification in the various substrate transport systems in the process of carcinogenesis is under active investigation. Therefore, studies directed at an understanding of transport systems in mammalian cells and changes in their structure or function with carcinogenesis will contribute to the knowledge required to document these changes and potentially to take advantage of the changes to develop specific drugs to control cancer cells.

Proposed Course: To continue to prepare photosensitive analogues with different properties to optimize specificity and reactivity of probes to aid in the identification and isolation of the nucleoside and amino acid transport systems. Techniques are being perfected using nucleoside transport in red cell sealed ghosts as a model system in which recognition and reconstitution of the transport system in liposomes will provide a model from which to proceed to the systems in normal and neoplastic cells. Further studies on the implications of the lipid alteration in transformed cells will involve other tumor cells, including several temperature sensitive lines.

Date Contract Initiated: June 26, 1974

Current Annual Level: $153,577

SOUTHERN RESEARCH INSTITUTE (NO1-CP-55721)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Director: Dr. Donald L. Hill

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: To synthesize or otherwise acquire six designated carcinogens labeled with carbon-14 and to study the metabolic pathways of these compounds in rats in attempts to determine their mechanisms of action.

Major Findings: Enzymatic activity leading to the irreversible binding of radioactivity from [1,2-^{14}C]1,2-dibromoethane to proteins is present in rat liver microsomes. The enzyme requires NADPH, is inducible by phenobarbital, and is inhibited by several sulfur-containing compounds. Studies on the tissue distribution and binding to macromolecules by radioactivity from [1,3-^{14}C] 1,2-dibromo-3-chloropropane, [methylene-^{14}C]4,4'-methylenebis (N,N-dimethylaniline), [carbonyl-^{14}C]4,4'-bis(dimethylamino) benzophenone, and

[4-methyl-^{14}C]2,4,6-trimethylaniline have been accomplished. For [^{14}C] 1,2-dibromo-3-chloropropane, a reaction catalyzed by rat liver microsomes, but not requiring NADPH, has been found. The product may be formed by hydrolysis of one of the halide groups. In addition, [^{14}C] 1,2-dibromo-3-chloropropane is a substrate for glutathione transferase. A urinary metabolite of [^{14}C] 4,4'-bis(dimethylamino)benzophenone has been tentatively identified as a didemethylated derivative.

[4-Methyl-^{14}C] 2,4,6-trimethylaniline was prepared by a procedure involving methylation of 2,6-dimethylaniline with [^{14}C]methyl iodide and rearrangement of the product by heating. A two-step synthesis of 4-chloro-2-methylaniline applicable to ^{14}C-labeling of the methyl group was demonstrated to give an overall yield of 15%.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the mechanism of action of carcinogens present in the human environment would allow steps to be taken to inhibit formation of active metabolites or to induce detoxifying enzymes. Since active metabolites of carcinogens quite likely become bound to tissue macromolecules, inhibitors of reactions leading to binding would be candidate anticarcinogens.

Proposed Course: (1) To prepare [^{14}C]4-chloro-2-methylaniline and study its tissue distribution and binding to macromolecules, (2) to determine the nature of urinary and fecal metabolites of the six carcinogens in rats, (3) to continue investigations on the enzymes involved in carcinogen metabolism and activation, and (4) to determine the mechanism of binding of activated metabolites to macromolecules.

Date Contract Initiated: June 30, 1975

Current Annual Level: $93,000

TEXAS, UNIVERSITY OF (at Houston) (NO1-CP-55604)

Title: Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Charles R. Shaw

Project Officer (NCI): Dr. Paul Okano

Objectives: (1) To further evaluate and improve the short-term human lymphocyte culture assay for measuring aryl hydrocarbon hydroxylase (AHH) activity in man, by studying a number of parameters to determine their effect on cell growth and enzyme activity. (2) To collaborate with the group at Roswell Park Memorial Institute in order to determine the basis for seasonal variation in AHH activity reported by them.

Major Findings: (1) Many parameters of the lymphocyte AHH assay were examined and the results are as follows: a) The reproducibility of the AHH assay was greater using freshly obtained fetal calf serum rather than ∿6-months frozen serum. b) Both F13 and RPMI 1640 culture media support approximately equal cell growth and AHH inducibility ratios. A 10% higher protein content and 10-15% lower AHH specific activity was obtained with RPMI medium. c) Heparin increased control and induced AHH activity 50-100% with little change in the inducibility ratio. With 60 units heparin per ml, cell growth increased 10% and protein content 20%. d) Time studies indicated that cell growth increased markedly between culture days 3 and 4, but AHH specific activity decreased. In studies using 4 subjects, more reproducible AHH results were obtained with 3-day cultures with a 24-hour induction period than with 4-day cultures. e) In a comparison of hydrocarbon inducers, lower cell growth was observed in the presence of 3-methylcholanthrene (3-MC) than in its absence, while no effect on cell growth was observed with benzanthracene (BA). (2) Duplicate blood samples were obtained in Houston and Buffalo and were cultured and assayed in both laboratories. No significant difference in cell growth or mean AHH activities, both uninduced and induced, was observed between the Buffalo specimens and the Houston specimens.

Significance to Biomedical Research and the Program of the Institute: Accurate measurement of AHH activity and the determination of its genetic control and variation in humans should allow the testing of the relationship of AHH activity to an individual's susceptibility to certain chemically-induced cancers and may provide a method for the detection of people highly susceptible to these cancers.

Proposed Course: Use of the method in studying human populations is now considered feasible. Efforts will continue to further improve reproducibility. Studies of subjects with recently diagnosed cancers, particularly bronchogenic carcinoma and squamous cell carcinoma of the nasopharynx, are underway.

Date Contract Initiated: August 15, 1974

Current Annual Level: $78,750

THERMO ELECTRON CORPORATION (NO1-CP-45623)

Title: Development and Application of Methods for Analysis of N-nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. David H. Fine

Project Officer (NCI): Dr. Larry Keefer

Objectives: The objective of this project was the development and application of specific analytical methods for selected N-nitroso compounds and their precursors for which suitable methodology did not then exist. Also to be pursued was the development and application of methods for determining total concentration of N-nitroso compounds and their precursors in foodstuffs and other

environmental mixtures. A suitable combination of analytical procedures has now been developed, and widespread application has begun so that the environmental distribution of N-nitroso compounds and their precursors is being investigated.

Major Findings: Development completed now.

Significance to Biomedical Research and the Program of the Institute: Analysis of certain N-nitroso compounds can now be carried out routinely.

Proposed Course: Complete in June, 1977.

Date Contract Initiated: June 18, 1974

Current Annual Level: $92,980

VANDERBILT UNIVERSITY (NO1-CP-65730)

Title: Non-Histone, DNA-Binding Proteins in Chemical Carcinogenesis

Contractor's Project Director: Dr. Lubomir S. Hnilica

Project Officer (NCI): Dr. Rufus Day

Objectives: 1) To develop selective large scale method for isolation of chromosomal non-histone DNA binding (NP) proteins from rat liver and hepatomas, subfractionate the DNA-binding NP proteins into individual protein species; 2) to determine their primary sequences in order to permit the construction of interaction models with DNA; 3) to characterize the DNA binding sites of tissue-specific NP proteins; 4) to localize the tumor-specific NP-DNA complexes by fluorescent antibody and horseradish peroxidase bridge techniques in order to detect malignant and premalignant cells in tissue sections; and 5) to investigate correlations between the tumor-specific DNA-binding proteins and the biological action sites of chemical carcinogens.

Major Findings: A new, relatively rapid method for the isolation of immunologically tissue-specific chromosomal nonhistone proteins was developed. This method employs removal of the bulk of chromosomal nonhistone proteins by extraction of chromatin with buffered 5 M urea and fractionation of the resulting residue by hydroxylapatite chromatography. Potassium phosphate buffer was found to be superior to the conventional sodium phosphate method. The tissue-specific fraction NP was further fractionated by gel filtration on Sephadex G-100 into low molecular weight and high molecular weight (30,000 and higher) proteins. The immunological activity was found associated with the high molecular weight proteins. To enhance the solubility of NP proteins in the absence of DNA, several polyanions were tested. It was found that dextran sulfate substantially increased the solubility of NP proteins without affecting their antigenic properties.

Because of their excessive aggregation, sodium-dodecyl sulfate must be added to the media used for electrophoretic separation of chromosomal nonhistone proteins. A method was developed for the removal of this detergent from electrophoretically separated NP proteins. This technique results in complete restoration of immunological properties of the NP proteins. Preparative electrophoresis of the NP proteins revealed that all the antigenic activity of this fraction is contained in two protein bands corresponding to molecular weight of approximately 60,000-70,000 daltons.

The horseradish peroxidase bridge localization of the NP proteins was performed in hepatomas and normal liver. The antiserum exhibited highly selective nuclear localization.

Chromatins from embryonic and regenerating rat liver (24-48 hours after hepatectomy) were found to fix the complement in the presence of Novikoff hepatoma antisera. Immunoabsorption experiments indicated the presence of at least two kinds of specific antibodies. One oriented against antigens common to highly proliferative tissues and the other oriented against antigens specific for malignant tumors. In in vitro, transcription of isolated chromatin was significantly inhibited by the addition of homologous antisera to the assay mixtures.

Significance to Biomedical Research and the Program of the Institute: The process of carcinogenesis frequently changes the transcriptional controls of individual genes. This leads to the expression of a new cellular phenotype, characteristic for the transformed cell. Work supported by this contract demonstrated that carcinogenesis changes the immunological and biochemical specificity of a small group of chromosomal nonhistone proteins (NP). This change was detected in all tested experimental malignancies and points to a change in the transcriptional controls of transformed cells. It is anticipated that the altered antigenic properties of transformed cells can be utilized for immunochemical detection of cancerous cells in sectioned tissues and biopsies. If the tissue-specific chromosomal nonhistone proteins are a part of the cellular gene-regulatory mechanism, detailed knowledge of these macromolecules may lead to new approaches to the control and prevention of cancer in man.

More specifically, the contracted research should lead to improved histochemical diagnosis of malignant and precancerous cells, identification of protein species responsible for the activation and perpetuation of cancerous phenotype, identification of DNA segments (or genes) responsible for malignancy, and, finally, the elucidation of transport mechanisms by which chemical carcinogens are incorporated into cellular genome.

Proposed Course: Using the preparative gel electrophoresis, we will prepare small amounts of the tissue-specific NP antigen for initial characterization. The chemical properties will then be used for developing a large-scale preparative method for this protein. Once available in sufficient quantities, the plan is to determine its amino acid sequence and study its interactions with homologous DNA. It is anticipated that the DNA loci interacting with the

tumor-specific NP antigen will lead to a gene cluster responsible for malignant transformation. Ultimately, a study will be made of the roles of NP proteins (these may be different from the tissue-specific NP antigen) in the mechanism of action of metabolically activated chemical carcinogens. In perspective, the studies will correlate the phenotypic changes of normal and malignant cells to the altered transcriptional controls resulting from the process of carcinogenesis.

Date Contract Initiated: February 20, 1976

Current Annual Level: $96,547

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-33220)

Title: Studies of Alterations Produced by Chemical Carcinogens in Viral and Cellular Gene Expressions

Contractor's Project Directors: Dr. Michel Revel
Dr. Uriel Littauer
Dr. Ernst Winocour
Dr. Haim Aviv

Project Officers (NCI): Dr. Harry Gelboin
Dr. Dolph Hatfield

Objectives: (1) To investigate the molecular mechanisms by which chemical carcinogens enhance viral-induced cell transformation. (2) To examine the effects of chemical carcinogens on cell-virus recombination events which occur during virus infection. (3) To study the effect of carcinogens on the expression of tumor viral genes.

Major Findings: (1) The techniques have been refined to enable a quantitative assessment of effect of chemical carcinogens on the recombination events between viral and the host genomes. (2) It was established that the recombination events which insert host DNA into the substituted SV40 DNA occur at preferred sites on the cellular genome, and that an exogenous SV40 genome can recombine with integrated SV40 genes in transformed permissive monkey cells. (3) A methodology to measure SV40 tumor antigen 90,000 and 17,000 MW polypeptides and T-ag mRNA has been developed to the stage where chemical carcinogens' effect on this viral-transformed gene can be studied. (4) Many carcinogens alkylate guanine at position 7; experiments demonstrate that 7-methylguanosine inhibits SV40 late protein synthesis in infected cells, possibly by competing with the methylated cap structure of nRNA. (5) The pattern of C-type viral RNA synthesis was established using a novel technique of hybridization between pulse-labeled RNA and excess amounts of DNA complementary to viral RNA. (6) Most of $tRNA^{Phe}$ in mouse neuroblastoma was found to lack modified base peroxy Y, while the normal brain tissues contain a fraction with incomplete peroxy Y-base.

Significance to Biomedical Research and the Program of the Institute: During induction of cell transformation, chemical carcinogens may interact with genetic information of viral or cellular origin. Elucidation of the molecular mechanisms by which chemical carcinogens and their metabolites affect integration and expression of tumor virus genome is important for understanding co-carcinogenesis.

Proposed Course: In the coming year, the nature, specificity, and extent of recombination between tumor viruses and the host genome leading to malignant transformation will be explored. Furthermore, the effect of chemical carcinogens on this process will be investigated. The effect of carcinogens on the expression of viral genes will be studied, particularly the effect on SV40 early and late genes as well as C-type viral genes. The hypothesis that alkylating chemical carcinogens interfere with viral protein synthesis or mRNA metabolism, favoring in this way transformation events over lytic viral development, will be investigated. The relationship between the lack of peroxy Y base and tumors will also be further examined, and the enzymatic pathway leading to the production of peroxy Y modification will be studied.

Date Contract Initiated: June 1, 1973

Current Annual Level: $286,808

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-02217)

Title: Study of the Role of Enzyme Induction in Chemical Carcinogenesis

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry Gelboin

Objectives: To clarify the role of polycyclic metabolism in carcinogenesis.

Major Findings: (1) Identification of Mutagenic Metabolites of Benzo(a) pyrene in Mammalian Cells - The mutagenicity of benzo(a)pyrene and 15 of its derivatives, which included phenols, the benzo(a) pyrene-4, 5-epoxide (the K-region epoxide), dihydrodiols, two isomeric 7,8-diol-9,10-epoxides, a 6-methyl derivative, and a 6-hydroxymethyl derivative, were tested with Chinese hamster V-79 cells by resistance to ouabain or 8-azaguanine. Since V79 cells do not metabolize polycyclic hydrocarbons, mutagenesis was tested both in the presence and absence of benzo(a)pyrene-metabolizing normal golden hamster cells. All the tested phenols, 4,5-diols, trans-9,10-diol, 6-methyl, and 6-hydroxymethyl derivatives of benzo(a)pyrene showed little or no mutagenicity for both genetic markers. The (±)7α,8β-dihydroxy-9α, 10α-epoxy-7,8, 9,10-tetrahydrobenzo(a)pyrene and K-region 4,5-epoxide exhibited similar and moderate mutagenicity in the absence of benzo(a)pyrene-metabolizing cells, but the (±)7α,8β-dihydroxy-9β, 10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene showed a 2000- and 270-fold higher mutation frequency for ouabain and 8-azaguanine resistance, respectively, than did the K-region 4,5-epoxide. The trans-7,8-diol which was not mutagenic in the absence of benzo(a)pyrene

metabolizing cells was more mutagenic than benzo(a)pyrene after metabolism and mutagenesis by trans-7,8-diol in these cells was inhibited by 7,8-benzoflavone, an inhibitor of mixed-function oxidases.

(2) Mutagenesis and Transformation of Normal Cells by Chemical Carcinogens - To elucidate the mechanism of carcinogenesis, it is important to determine the genes which may be involved in cell transformation. The frequency of cell transformation and mutagenesis of specific loci can both be tested in vitro. In normal diploid cells, the ratio between transformation and mutation of a specific locus is small (about 10) or large (more than 10). Mutation and transformation frequencies were determined in primary cultures of normal golden hamster embryo cells treated with the carcinogenic hydrocarbon benzo(a) pyrene and its metabolite 7,8-trans-dihydrodiol. The frequency of transformed colonies has been compared with the frequency of mutation for ouabain resistance, a mutation associated with the surface membrane Na^+/K^+ ATPase. This mutation was chosen because of its low spontaneous frequency and the ease with which it can be detected in normal diploid cells. The results have shown that benzo(a)pyrene and its 7,8-dihydrodiol can induce both transformation and mutagenesis for ouabain resistance in normal diploid cells. The ratio between transformation and mutagenesis for ouabain resistance was about 20:1 with both hydrocarbons. Since ouabain resistance is presumably due to mutation at one locus, it can be suggested that transformation has a target equivalent to about 20 such genes. There may thus be this number of the same or different genes, the mutation of any one of which is the single hit required for transformation. The studies suggest that cell transformation is due to a mutation and that this mutation can occur in one out of a small number of the same or different genes.

(3) Independent Regulation of Two Types of Aryl Hydrocarbon (Benzo(a)pyrene) Hydroxylase in Mammalian Cells - Aryl hydrocarbon (benzo(a)pyrene) hydroxylase induced by dibutyryl cyclic AMP (dcAMP), plus aminophylline (AHH I) can be distinguished from the hydroxylase induced by benz(a)anthracene (AHH II) by its lower Km for benzo(a)pyrene. Treatment with the combination of benz (a) anthracene and dcAMP plus aminophylline induced both AHH I and AHH II activities. After optimal induction of AHH II activity by benz(a)anthracene, the addition of dcAMP plus aminophylline gave an induction of AHH I. Although AHH I activity declined to an almost basal level 24 h after treatment with dcAMP plus aminophylline, the addition of benz(a)anthracene prevented this decline. Inducibility by dcAMP plus aminophylline or by benz(a)anthracene varied in different cell lines. Some of the cell lines were induced by both substances, while other lines were inducible only by benz(a)anthracene, and a third cell type was not inducible by either. Selection for resistance to benz(a)pyrene of a cell line inducible by both compounds resulted in a fourth cell type which was more inducible by dcAMP plus aminophylline than by benz(a)anthracene. The results suggest that there is an independent regulation of hydroxylase AHH I and AHH II and that the induction of these two enzyme activities is determined by different genetic controls.

Significance to Biomedical Research and the Program of the Institute: An understanding of these genetic and biochemical processes is necessary to understand carcinogenesis in humans.

Proposed Course: To further clarify the relationship between the AHH enzyme system, the metabolism of carcinogenic hydrocarbons, and malignant cell transformation.

Date Contract Initiated: May 26, 1970

Current Annual Level: $189,355

WISCONSIN CLINICAL CANCER CENTER/UNIVERSITY OF WISCONSIN (NO1-CP-65734)

Title: Induction of Aryl Hydrocarbon Hydroxylase in Cultured Human Lymphocytes

Contractor's Project Director: Dr. Gottfried Kellermann

Project Officer (NCI): Dr. Paul Okano

Objectives: 1) To evaluate further the reproducibility of AHH induction in cultured human lymphocytes, and 2) to relate the relative AHH activity and inducibility in cultured lymphocytes to the in vivo oxidation rates of certain model drugs which resemble in their metabolism that of benzo(a)pyrene and other carcinogens.

Major Findings: An excellent correlation has been obtained between the in vitro AHH test in lymphocytes and the oxidative metabolism of several drugs in vivo. The data indicate that the measurement of plasma elimination rates of antipyrine is an accurate index of an individual's relative AHH activity and inducibility. As an alternative AHH test it opens the possibility of testing the hypothesis that individuals with lung cancer have higher epoxidation rates.

Significance to Biomedical Research and the Program of the Institute: It has been widely accepted that most chemicals which are carcinogens have to be enzymatically activated to become active carcinogens. There is a possibility that people differ genetically with respect to these activating enzymes and consequently can be either highly susceptible or resistant to the action of certain carcinogens. Therefore, it is of great importance to develop tests which can identify individuals who have either high or low epoxidation rates. Since these varying epoxidation rates are very likely under genetic control, it opens the possibility of identifying people who would be highly susceptible to certain chemically-induced cancers.

Proposed Course: The correlation between the in vitro and in vivo AHH test has been established in the first phase of the contract. In the second phase, the new in vivo test will be evaluated for its usefulness in patients with cancer of the lung. The mode of inheritance of AHH activity will also be re-evaluated using the new in vivo test.

Date Contract Initiated: August 18, 1975

Current Annual Level: $98,500

WISTAR INSTITUTE (NO1-CP-55655)

Title: Significance of Mutagenesis in Carcinogenesis

Contractor's Project Director: Dr. Leila Diamond

Project Officers (NCI): Dr. Rufus Day
Dr. Andrew C. Peacock

Objectives: To investigate the possibility that chemical carcinogens act by inducing somatic mutations at specific sites on the genome concerned with the regulation of growth.

Major Findings: A cell system(s) in which both transformation and mutation induced by chemical carcinogens can be assayed under the same conditions is being developed. After three successive clonings, three subclones with a flat morphology and low saturation density were isolated from NTG, a 6-thioguanine-resistant BALB/c 3T3 clone obtained from K. Jha and H. Ozer. The optimal conditions for selecting ouabain-resistant and thioguanine-sensitive mutants of these clones have been worked out. The susceptibility of the clones to chemically-induced transformation is being determined. The thrice-cloned sublines also were isolated from the original BALB/c 3T3 cell line. Their suitability in transformation assays is being compared to that of a clone recently isolated by Dr. T. Kakunaga.

Significance to Biomedical Research and the Program of the Institute: The eventual control of chemical carcinogenesis will be either through the identification of carcinogens and their elimination from the environment or through understanding, and thereby controlling, their mode of action. Thus, an understanding of the relationship between carcinogenesis and mutagenesis is important for both practical and theoretical reasons. Our approach to investigating this relationship is to obtain a system in which both events can be measured and compared under similar conditions.

Proposed Course: Several subclones of wild-type and 6-thioguanine-resistant BALB/c 3T3 cells are being characterized with respect to growth properties and susceptibility to transformation induced by several types of chemical carcinogens. The clones most suitable for quantitative transformation assays will be selected and the frequency of transformation and mutation by carcinogens in the same population under identical conditions will be measured. Both forward and reverse mutations of the 6-thioguanine-resistant clone will be scored.

Date Contract Initiated: June 27, 1975

Current Annual Level: $116,750

YALE UNIVERSITY (NO1-CP-55673)

Title: Selection and Propagation of Monosomic and Haploid Cell Lines

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officer (NCI): Dr. John P. Bader

Objectives: To isolate monosomic, partially monosomic, and haploid mouse cells and to perform a somatic cell genetic analysis of in vitro chemical carcinogenesis. Teratomas are being used for the production of monosomic and partially monosomic cell lines because they are chromosomally stable near the diploid level and their genetic constitution can be manipulated as required. Spermatids are being used to produce haploid cell lines.

Major Findings: Highly purified spermatid preparations are routinely produced by elutriation. Fusion of mouse spermatids with HeLa cells results in the synthesis of DNA by the spermatid nuclei. Several lines of mouse embryonal carcinoma cells monosomic for either chromosome 4 or 11 have been derived from OTT 6050, a mouse teratocarcinoma. Single or small numbers of chromosomes have been transferred to recipient cells by fusion with subnuclear particles (microcells). A line of fibroblasts for use in the carcinogenesis project with a stable karyotype of 41 chromosomes has been established from a $B6D2F_1$ (C57BL/6 x DBA/2) teratoma. A hypoxanthine phosphoribosyltransferase deficient ($HPRT^-$) subline has been derived from it.

Significance to Biomedical Research and the Program of the Institute: Well-characterized monosomic or haploid cell lines will greatly increase the sensitivity of assays for chemically induced transformation. A somatic cell genetic analysis of transformation will determine whether it is due to mappable mutations or to epigenetic events and whether a single lesion or multiple lesions can give rise to the transformed phenotype. Mice of novel genetic constitution, especially those which have incorporated a small piece of a foreign genome, will allow identification of genetic elements which regulate specific epigenetic events.

Proposed Course: A number of teratoma cell lines will be analyzed in detail to establish their pattern of monosomy. Hybrids of mouse spermatids with human cells from which the human chromosomes are likely to be segregated will be made. Activation of spermatids by fusion with microcells will also be tried. Work will be continued on the production of enzyme-deficient mice by implanting HPRT embryonal carcinoma cells into mouse blastocysts and scoring the resulting animals for incorporation of these cells into the germ line.

Date Contract Initiated: January 18, 1975

Current Annual Level: $115,244

SUMMARY REPORT

INTERDISCIPLINARY PROGRAMS AND RESOURCES
OPERATIONAL UNIT

July 1, 1976 through September 30, 1977

The contracts in the Interdisciplinary Programs and Resources Unit consist of service projects as well as special research programs. They include three large interdisciplinary programs covering several areas of carcinogenesis research; animal and chemical resources; and projects relating to multi-disciplinary efforts that do not belong in other program areas.

Interdisciplinary Projects

The three interdisciplinary efforts are (1) the NCI program at the Frederick Cancer Research Center managed by Litton Bionetics, Inc. (NO1-CO-25423); (2) an interagency agreement with the Energy Research and Development Administration for research carried out at the Oak Ridge National Laboratory (YO1-CP-50200); and (3) the program of the Eppley Institute for Research on Cancer at the University of Nebraska (NO1-CP-33278).

Frederick Cancer Research Center (FCRC)

The FCRC has as its primary objective the development of a unique national resource capable of initiating and evaluating a broad range of approaches to effectively identify human carcinogens. Toward this end, the Center employs a coordinated interdisciplinary approach which consciously integrates basic and applied research. Research programs have been initiated which address various facets of the following general areas: identification of carcinogenic or co-carcinogenic environmental chemicals; investigation of host-chemical interactions; and attempts to elucidate mechanisms of carcinogenesis to permit the manipulation of factors which will block or reverse tumor formation.

Among the major research findings resulting from this program are the following:

- Studies concerned with the mechanism of bile acid activation of chemical carcinogens have shown that lithocholic acid (LA) affects the metabolism of 2-anthramine (2-AA) and benzo[a]pyrene (b[a]P). Substrate specificity appears to be critical in the metabolism of physiological lethocholic acid derivatives by human intestinal microflora;

- Of the 21 compounds tested under the Center's Bioassay Research Program, azobenzene appears to be carcinogenic in rats, causing a wide variety of sarcomas of the spleen and other abdominal organs;

- A major advance in the preparation of derivatives of carcinogenic polycyclic aromatic hydrocarbons, resulting in a substantial decrease in the number of steps required for their synthesis as compared with conventional routes;

- Standardization of a hamster embryo cell carcinogenesis bioassay to the extent that almost complete correlation (91%) was obtained when coded test results of 88 reference chemicals were compared with known activity;

- The drafting of guidelines for the safe handling of 11 known or suspected chemical carcinogens;

- The development of a system for the growth and maintenance of normal human breast epithelial cells in culture "free" from contaminating fibroblasts which have been used to study the binding of DMBA to DNA;

- It was concluded through experiments involving comparisons of the excision of DNA damage that carcinogenic potency probably does not reside in an ability to introduce intrinsically irreparable damage into cellular DNA. Alkylating agents of different carcinogenic potencies were found to exhibit different selectivities in their reactions with DNA, with the more carcinogenic agents distributing themselves more randomly;

- A significant incidence of induced tumors in laboratory rats due to the combination of amine and nitrite in their diets, indicating a large measure of formation of N-nitroso compound in the stomach.

ERDA-NCI Interagency Agreement (Oak Ridge National Laboratory)

Providing an interdisciplinary research approach to solving problems of chemical carcinogenesis continues to be the major objective of this program. Research includes probes into respiratory carcinogenesis, tumor cell membrane and DNA repair, in vitro carcinogenesis and metabolism, and activation of chemical carcinogens. Major contributions resulting from this program include: (a) Results from seven different NO_2-carcinogen exposure groups (80 hamsters per group) show that NO_2 does not measurably enhance pulmonary carcinogenesis induced by BaP; (b) Progress made in applying the focus assay method to problems encountered in cell culture development; (c) The development of S'-nucleotidase as a reliable quantitative marker for the preparation and analysis of isolated human tumor cell membranes, with 95% localization; (d) Results showing that only the 7,8 dio-9,10 epoxy benzo[a]pyrene isomers elicit damage to DNA which is repairable; (e) The establishment of a correlation between growth in soft agar and tumorigenesis and neoplastic transformation in cells derived from carcinogen exposed organ cultures.

Eppley Institute for Research in Cancer (Nebraska University)

The project's principle objectives are the bioassay of suspected chemical carcinogens, definition of the biological and chemical mechanisms of the carcinogenic process, and the analysis of trace levels of environmental carcinogens.

Among the major findings: New hydrazine analogues were shown to be carcinogenic in laboratory animals, including β-phenylethylhydrazine sulfate; succinic acid, 2,2-dimethylhydrazine, N-acetyl-4-(hydroxymethyl) phenylhydrazine, an acetylated derivative of 4-hydroxymethyl-phenylhydrazine, an ingredient of the commonly eaten, cultivated mushroom Agaricus bisporus, and N-methyl-N-formylhydrazine. Three hydrazine derivatives—1,1-dimethylhydrazine; methylhydrazine, and trimethylhydrazine HCl—induced significant incidences of tumors in laboratory animals. Highlighted in the bioassay is the induction of a systemic neoplastic effect by 3 nitrosamines; injected nitrosamines (e.g., methylamylnitrosamine) caused acute inhibition of [^{3}H]thymidine uptake by epithelial DNA of the rat esophagus. The pancreatotrophic effect of N-nitrosobis(2-oxopropyl)amine (BOP), N-nitrosobis-(2-hydroxypropyl)amine (BHP) and N-nitrosobis(2-acetoxypropyl)amine (BAP) was found to be directly related to in vivo formation of N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) and hamsters were shown to be as sensitive as rats in tests of acute toxicity of various chlorinated pesticides. Other studies on the mechanism of carcinogenesis have developed an improved synthetic route for the production of agaritine, a naturally occurring hydrazine compound found in the mushroom family. Another mushroom extract, gyromitrin, produces the carcinogen methylhydrazine in the mouse stomach. A gas-liquid chromatographic method for the estimation of methylhydrazine and other hydrazines in an aqueous environment has been developed. Pyridoxine hydrochloride (vitamin B_6) was shown to reverse the toxic action of 4 substituted hydrazines.

Projects in In Vitro Carcinogenesis

In another group of projects funded from this program area, the major objectives are aimed at development and validation of in vitro methods as prescreens for carcinogenesis bioassays. A contractor at Columbia University (NO1-CP-23234) found the tumor-promoting agent, 12-O-tetradecanoyl phorbol-13-acetate (TPA) to be a potent inducer of plasminogen activator production in cell culture which may provide a rapid bioassay for promoting agents and an in vitro system for making clear their mechanism(s) of action. It also appears that plasminogen activator production is not a reliable criterion for predicting the tumorigenic potential of rat liver epithelial cell cultures, although growth in soft agar suspension and certain cytologic properties are useful markers for the transformation of epithelial cultures.

Studies have continued at Salk Institute (NO1-CP-33405) to determine the kinds of changes accompanying chemical transformation of cells by focusing on growth control mechanisms of chemically-induced tumors. To this end,

work has concentrated on understanding the changes in growth control that occur when 3T3 cells are transformed by benzo[a]pyrene. This transformation has been found to be accompanied by a decreased utilization of serum growth factors which contributes to the tumorigenicity of these cells in vivo and which is partly accounted for by a decreased affinity of the receptor sites for the growth factor.

Other Projects

Other projects, not relating to any specific segment, but of relevance and importance to the entire Carcinogenesis Program, are also supported by this program area. At the Children's Hospital of Philadelphia (NO1-CP-65803) a study concerning oncogenesis and other late effects of cancer therapy is being conducted. The program—in cooperation with 11 pediatric oncology centers (CICI)—seeks to establish, among other things, a central data repository of unusual problems of childhood cancer, such as the late effects of treatment, the incidence of second malignant neoplasms (SMN) and their relationship to treatment, the offspring of survivors and the association of congenital anomalies in patients with various tumor types and siblings.

Chemical Resource Activities

The Chemical Repository is located at the IIT Research Institute (NO1-CP-55646), in a facility built to ensure safe handling of carcinogenic materials. There are now over 160 compounds available for distribution to NCI contractors, with over one thousand samples having been distributed to researchers in the last year. Under Basic Ordering Agreements (BOA's), IIT Research Institute (NO1-CP-65745), Midwest Research Institute (NO1-CP-65739), Southern Research Institute (NO1-CP-65740), and Stanford Research Institute (NO1-CP-65741) are synthesizing compounds for the Repository in laboratories designed for safe handling of carcinogens.

The analytical chemistry services of the Midwest Research Institute (NO1-CP-23270) were utilized for 50 batches of chemicals, representing 20% of the total number of studies conducted since June 1972.

The synthesis of isotopically-labeled N-nitroso compounds has been completed at Stanford Research Institute (NO1-CP-55672), but continues at the University of New Hampshire (NO1-CP-55675). Spectral studies of nitrosamines by the latter contractor indicate that further research in interactions of the nitrosamine function will be fruitful. The Stanford Research Institute (SRI) contract was permitted to terminate during this fiscal year, having accomplished the goal of proving that a market for isotopically-labeled N-nitroso compounds exists. The compounds synthesized by SRI are now commercially available through New England Nuclear. This project is an example of how resource development can be used to indicate markets which can then be picked up by commercial interests.

The contract with Thermo Electron Corporation (NO1-CP-65822), to produce interfaces for the Thermal Energy Analyzer to be used in conjunction with high pressure liquid chromatography, has been completed. The interface greatly increases the ability of the Thermal Energy Analyzers in detecting N-nitroso compounds.

The production and characterization of powder fractions and dispersions for respiratory studies from the IIT Research Institute (NO1-CP-33296) will continue through FY 77, when remaining stock will be turned over to the Chemical Repository. The University of Chicago (NO1-CP-33385) and the Midwest Research Institute (NO1-CP-33387) are continuing programs to synthesize polycyclic hydrocarbon derivatives.

Animal Resource Activities

Medizinische Hochschule Hannover (NO1-CP-12148) continues the development of the European hamster (*Cricetus cricetus* L.) as an animal model for the induction of respiratory tract tumors morphologically similar to tumors found in man. An English language anatomical atlas of the animal is in press. Testing of known carcinogens, bronchoscopic techniques, and the development of a pancreatic tumor model are ongoing. Harlan Industries, Inc. (NO1-CP-55647) is producing AxC inbred and Sprague-Dawley random-bred strains of aged male rats for use in prostate cancer research.

Biology and Immunology

The University of California's (at San Diego) (NO1-CP-33403) investigation of the relationship between carcinogenic events and alpha-fetoglobulin production has been completed; the University of Utah's (NO1-CP-45600) studies on the relationship between carcinogenesis and immunosuppressants are also completed, with the exception of final statistical analyses.

CONTRACT NARRATIVES

INTERDISCIPLINARY PROGRAMS AND RESOURCES OPERATIONAL UNIT

July 1, 1976 through September 30, 1977

CALIFORNIA, UNIVERSITY OF (at San Diego) (NO1-CP-33403)

Title: Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogens

Contractor's Project Director: Dr. Stewart Sell

Project Officer (NCI): Dr. Tibor Borsos

Objectives: To develop a sensitive new method to study the relationship between the induction of primary hepatomas and the appearance and significance of fetoglobulins.

Major Findings: Gestation: The tissue sites of synthesis of AFP by the rat during gestation and hepatoma growth were determined by specific incorporation of a radiolabeled precursor into AFP by short-term tissue cultures in vitro. During gestation, AFP was produced by the yolk sac, the fetal liver and in small amounts by the fetal gastrointestinal tract; there was no synthesis by maternal tissues. During growth of a transplantable hepatoma, only the hepatoma tissue produced detectable AFP. The tissue origin of tumors that produce AFP in the adult is closely related to fetal tissue production. Thus, AFP is produced by yolk sac tumors (embryonal carcinomas) and hepatomas of adult rats. Transplantable yolk sac tumors of the rat may be produced by transplantation of embryonal tissue. The sera of rats bearing transplantable yolk sac tumors have elevated concentrations of AFP.

Hepatoma AFP Production and Effects of Therapy: Transplantable rat hepatomas may produce elevated serum concentrations of AFP. Sequential analysis of transplantable hepatomas over a two-year period demonstrated that in almost all instances the growth rate, morphology, chromosome composition, and plasma protein and AFP production by individual tumors remained constant. One subline of a diploid tumor demonstrated an extensive re-arrangement of its chromosomes which was associated with a significant acceleration of growth rate. In spite of this, AFP and plasma protein production did not change.

If a tumor does produce AFP, the serum concentrations of tumor-bearing rats may be used to follow the effects of therapy. For instance, following transplantation of hepatoma 7777 into syngenic rats, there is an exponential increase in the serum concentration of AFP, until the animals die of massive tumor. If the tumor is removed surgically, the serum AFP concentration will fall abruptly; re-elevations occur with growth of metastatic lesions

primarily in the lung; a continued decline to normal adult serum concentrations generally indicates complete removal of the tumor and absence of metastatic growth. Using this model, the effect of passive administration of anti-AFP on tumor growth and the effect of irradiation of the lung at the time of amputation of the primary tumor transplant site was determined. Passive transfer of goat anti-AFP at the time of tumor transplantation prevented tumor growth in some, but not all, rats. Irradiation to the lung following surgical amputation of the primary transplanted tumor is an effective means of inhibiting metastatic growth.

Effect of AFP on Immune Responses: One of the hypothesized functions of AFP is that it might protect the fetus from maternal immune attack. This hypothesis has been supported by observations that AFP may inhibit some in vitro responses of lymphoid cells. However, both in vivo and in vitro studies carried out during the last year failed to demonstrate a consistent immunosuppressive effect for AFP in both mice and rats. There were no significant differences in the effects of natural fluids containing over 100,000-fold differences in AFP concentrations on mitogen responses or mixed lymphocyte reactions. Very high concentrations of purified AFP partially inhibited primary in vitro responses to SRBC but had a 2-4 fold inhibitory effect on secondary responses to keyhole limpet hemocyanin conjugated to trinitrophenol. Purified albumin was also inhibitory in some systems. Therefore, although some effects on in vitro lymphocyte reactions may be produced by addition of AFP, these data do not support a general immunosupressive role for AFP. AFP may function locally at the fetal-maternal interface to inhibit immune responses but such a function should be reconsidered in view of the above findings.

Carcinogenesis: Early studies demonstrating elevations of serum AFP in rats treated with chemical hepatocarcinogens have been extended. Prolonged elevations were previously found after only short exposure of rats to subcarcinogenic doses of the carcinogen 2-FAA, but not to noncarcinogenic chemical analogues of 2-FAA. Further studies have shown that continued exposure to 2-FAA after a long period actually results in a decline in serum AFP concentrations. Later elevations (after 14-16 weeks) occur when frank hepatocellular carcinomas appear. In contrast to other carcinogens, exposure to the powerful hepatocarcinogen, diethylnitrosamine, is not associated with early serum AFP elevations. Elevations are only detected when approximately the total carcinogenic dose has been administered, i.e., high doses produce elevations after a few weeks, low doses, after a few months. These findings raise questions as to the significance of the early elevation of serum AFP found with other hepatocarcinogens. Additional data related to this point have been obtained from a study on the effect of aging on the serum AFP concentrations of a strain of mice which spontaneously develop hepatomas. No elevation with aging alone was found. Elevations only occurred when hepatomas developed. Thus, despite the obvious increasing risk for malignancy, no increase in AFP was noted.

Hepatocellular Injury: The effect of restitutive hepatocellular proliferation upon serum AFP concentrations was studied further using the model of galactosamine and CCl_4 hepatitis. Following injury, elevated serum AFP concentrations follow initiation of hepatocellular proliferation. The

serum concentrations of AFP of rats following galactosamine-induced liver cell necrosis accurately reflects the degree of preceding liver cell damage as determined by the degree of elevation of serum transaminases. In surviving animals, the serum AFP concentrations return to normal within 10 days to 2 weeks and are lower than the elevation caused by hepatocarcinogens. Thus, the degree and kinetics of elevation of serum AFP following necrotic injury and restitutive proliferation are different from the the effects of hepatocarcinogen exposure or hepatoma growth.

In Vitro Studies: The synthesis and release of AFP by fetal hepatocytes in vitro also demonstrates a link between AFP synthesis and the growth cycle. AFP and albumin are produced by arginine synthesizing fetal rat hepatocytes in vitro. AFP and hemopexin production apparently are coupled to proliferation of these cells, whereas albumin and hepatoglobin production are not. Drugs which block DNA synthesis do not block AFP synthesis, suggesting that AFP synthesis is not required for DNA synthesis. To date, G_1 and/or G_2 synthesis, with or without post-mitotic release, are alternatives which as yet have not been distinguished by the data. Combined immunofloures-cent and ^{3}HdT autoradiography studies of fetal rat hepatocytes cultures have confirmed previous kinetic data and shown directly differential regulation of albumin and AFP during the growth cycle. Cell-cycle synthesis times have not yet been determined. Fetal hepatocytes in the same topological location on the dish surface contain AFP and albumin. However, AFP is only demonstrable when the cells are in a growth phase, whereas albumin is present during the lag, logarithmic and stationary growth phases. During the latter part of logarithmic growth, approximately 10 percent of the AFP positive cells are also synthesizing DNA as determined by ^{3}HdT uptake, therefore it is certain that cycling cells produce AFP but the exact relationships with growth states are not yet clear. Further studies on the effects of hormones on AFP production and liver cell proliferation in vitro have been carried out. Primary monolayer cultures of adult, as well as fetal hepatocytes, have been reproducibly established. AFP production by adult hepatocytes in vitro is also associated with hepatocyte proliferation. In addition, it has been shown that adult liver cell cultures are able to metabolize the hepatocarcinogen 2-FAA. This system is now being developed for further studies on hepatocarcinogens in vitro.

Acute Phase Alpha$_2$ Macroglobulin: Studies have been carried out on rat accute phase α_2 macroblobulin ($AP\alpha_2M$). Rat $AP\alpha_2M$ was purified by affinity chromatography and a radioimmunoassay with a sensitivity of 0.020 µg established. Sera from normal adult rats contain 20-30 µg/ml, fetal rats 9,000-10,000 µg/ml and maternal rats 900 µg/ml at gestation. Induction of inflammatory injury is associated with an increase to 9,000-10,000 µg/ml in adult rats within 36 hours. Following experimental liver injury with CCl_4 or galactosamine or after partial hepatectomy, serum $AP^{\alpha}{}_2M$ and AFP are both elevated but with different kinetics; $AP^{\alpha}{}_2M$ elevations occur within 24 hours, AFP only after 2-3 days. It is concluded that synthesis of AFP and $AP^{\alpha}{}_2M$ are under separate control. In addition, $AP^{\alpha}{}_2M$ is not synthesized by proliferating adult hepatocytes as is AFP. $AP^{\alpha}{}_2M$ is not elevated after exposure to hepatocarcinogens or during the growth of transplantable hepatocellular tumors. The data so far obtained strongly suggest that production of $AP^{\alpha}{}_2$ is not

associated with tumor growth but that it is an acute phase reaction produced in response to inflammatory injury. In view of the anti-endopeptidase activity of these macroglobulins, it is suggested that $AP\alpha_2M$ functions to limit the extent of tissue inflammation.

Significance to Biomedical Research and the Program of the Institute: The experimental observations in regard to AFP production during tumor induction and growth provide evidence that serum AFP concentrations may be used to monitor the effects of tumor therapy as well as possibly to detect exposure to hepatocarcinogens prior to hepatoma development. Since a primary goal of the carcinogenesis program is to detect and prevent cancer in man, it is clear that the time is at hand when surveys of patients at risk for hepatocellular or embryonal cell tumors should be followed by serum AFP determinations. These findings assume greater significance when viewed in the light of an increasing incidence of hepatoma in the United States. In addition, studies on the control of expression of AFP during cell growth and hepatocarcinogenesis provide a mechanism for studying the early events in carcinogenesis.

Proposed Course: This is the terminal year of this contract.

Date Contract Initiated: June 28, 1972

Current Annual Level: $181,427

CHICAGO, UNIVERSITY OF (NO1-CP-33385)

Title: Synthesis of Derivatives of Carcinogenic Polycyclic Hydrocarbons

Contractor's Project Director: Dr. Ronald G. Harvey

Project Officer (NCI): Dr. Shen Yang

Objectives: To synthesize polycyclic hydrocarbon derivatives and metabolites to be available to the scientific community for in vivo and in vitro studies in carcinogenesis. These compounds are of special value in studying enzyme pathways in carcinogenesis.

Major Findings: (1) Preparative scale syntheses have been completed and samples submitted on the following compounds: *trans*-8,9-dihydroxy-8,9-dihydro-BA; *trans*-10,11-dihydroxy-10,11-dihydro-BA; *trans*-8,9-dihydroxy-*anti*-10,11-epoxy-8,9,10,11-tetrahydro-BA; *trans*-8,9-dihydroxy-*syn*-10,11-epoxy-8,9,10,11-tetrahydro-BA; 8-hydroxy-BA; 9-hydroxy-BA; *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-BP; *trans*-7,8-dihydroxy-*syn*-9,10-epoxy-7,8,9,10-tetrahydro-BP; *cis*-7,8-dihydroxy-7,8-dihydro-BP; BP-1,6-dione; BP-7,10-dione; BP-11,12-dione. (2) Research scale exploratory syntheses have been successfully completed on the following compounds: *trans*-10,11-dihydroxy-*anti*-8,9-epoxy-8,9,10,11-tetrahydro-BA; 10-hydroxy-BA; *trans*-1,2-dihydroxy-1,2-dihydro-BA; *trans*-3,4-dihydroxy-3,4-dihydro-BA; *trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydro-BA; *trans*-1,2-dihydroxy-*anti*-3,4-epoxy-1,2,3,4-tetrahydro-BA; *cis*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-BP; 3-^{2}H-BP; 7-^{2}H-BP; 7-hydroxymethyl-12-methyl-BA-5,6-oxide;

7-bromomethyl-12-methyl-BA; 12-bromomethyl-7-methyl-BA; 7,12-*bis*-(bromomethyl)-BA; 7-^{2}H-DMBA; 7-^{3}H-DMBA; *trans*-4,5,7-trihydroxy-4,5-dihydro-BP; *trans*-7,8-dihydroxy-9-keto-7,8,9,10-tetrahydro-BP; 7-methyl-BP. (3) Syntheses of the following compounds are in progress and anticipated to be complete in this contract period: 11-hydroxy-BA; 1-hydroxy-BA; 2-hydroxy-BA; 3-hydroxy-BA; 4-hydroxy-BA; 12-hydroxy-BA; *cis*-7,10-dihydroxy-8,9-epoxy-7,8,9,10-tetrahydro-BP; *trans*-3,4-dihydroxy-*syn*-1,2-epoxy-1,2,3,4-tetrahydro-BA; *trans*-1,2-dihydroxy-*syn*-3,4-epoxy-1,2,3,4-tetrahydro-BA; BP-7,8-dione; 6-methyl-BP. (4) Attempts to synthesize the following compounds have not been successful due to the exceptional instability of the synthetic intermediates: 1,2-dihydroxy-1,2,3,6-tetrahydro-BP (precursor of *trans*-1,2-dihydroxy-BP); 2-keto-1,2,3,6-tetrahydro-BP (precursor of 2-hydroxy-BP); BP-1,2-dione; BP-1,2-oxide. (5) All compounds and synthetic intermediates are thoroughly characterized by appropriate spectroscopic and chromatographic techniques including ultraviolet, infrared and nmr spectroscopy, mass spectra, melting point, microanalysis, tlc, and hplc. Selected compounds of particular biological importance are also submitted for X-ray crystallographic analysis. 270 MHz high resolution nmr spectroscopy has been utilized extensively to aid in structural assignment of isomeric products and elucidate details of stereochemistry and molecular structure.

Significance to Biomedical Research and the Program of the Institute: Recent evidence supports the theory that metabolic activation is essential to the carcinogenic activity of polycyclic hydrocarbons, and a diolepoxide of BP, (+)-7β, 8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-BP, which was synthesized in this program has been implicated as the active form of this carcinogen. The other known or suspected metabolites of carcinogens synthesized in this project are of urgent need in carcinogenesis research. It would be virtually impossible for any laboratory lacking specialized synthetic experience in this field to synthesize any one of these compounds. Thus, the combined contract, of which this project is part, puts the National Cancer Institute in the position of providing the essential compounds for experiments in chemical carcinogenesis which could not be carried out otherwise.

Proposed Course: Compounds for which syntheses have already been devised will be prepared in the required scale, and exploratory experiments to develop convenient syntheses of the other compounds sought will continue.

Date Contract Initiated: June 20, 1973

Current Annual Level: $100,000

THE CHILDREN'S HOSPITAL OF PHILADELPHIA (NO1-CP-65803)

Title: Oncogenesis and Other Late Effects of Cancer Therapy

Contractor's Project Director: Dr. Giulio J. D'Angio

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) Establishment of a central repository of unusual problems of childhood cancer such as the late effects of treatment, the incidence of second malignant neoplasms (SMN) and their relationship to treatment, the offspring of survivors and the association of congenital anomalies in patients with various tumor types and siblings; (2) prospective evaluation of neuropsychologic and intellectual functioning in a group of patients being treated for acute leukemia with cranial irradiation and intensive chemotherapy; (3) prospective evaluation of pulmonary function in patients treated with radiation to the lungs by spirometry and Xenon Scan prior to and at intervals from such treatment; (4) ascertainment of the host factors responsible for the development of second malignant neoplasms (SMN) in children as well as the treatments which are related to their development; (5) organization of a feasibility study to evaluate the extent of impairment and disability relating to specific treatment in a large group of survivors of childhood cancer; (6) continuation of the chromosomal analysis of long-term survivors of childhood cancer, such that temporary and permanent changes associated with chemotherapeutic agents and radiation therapy can be appreciated.

Major Findings: (1) An inventory of pediatric cancer patients (CICI) has been established at The Children's Hospital of Philadelphia utilizing the cooperation of eleven pediatric oncology centers. There have been 1,350 patients registered since July 1, 1975. (2) All new patients with leukemia and lymphoma (34) treated with cranial irradiation, intrathecal methotrexate and other drugs have been pre-examined by a neurologist and psychologist. There are six control children-patients with Wilms' tumor treated with abdominal (some with pulmonary) irradiation. Twelve have been retested at least one year later and, of these, two had some changes in verbal memory functions. Continued testing longitudinally and on more children is needed to determine the significance of these findings. (3) Additional patients with SMN were ascertained from the eleven cooperating pediatric centers so that we now have recorded a total of 135. Radiation was associated with 90 second malignant neoplasms, genetic disease accounted for 40, and both conditions were noted in 22. All pediatric cancers were seen as initial lesions, but Wilms' tumor and retinoblastoma were over-represented and leukemia and brain tumors were under-represented. Survival variation as well as tumor susceptibility may be responsible for this disproportion. Susceptibility was expressed in 25 patients with the genetic form of retinoblastoma, 7 with von Recklinghausen's disease, 4 with nevoid basal cell carcinoma syndrome and 4 with xeroderma pigmentosum. Osteosarcomas and chondrosarcomas were the most frequent SMNs. Embryonal tumors were rare as SMN and adult-type tumors (carcinomas) appeared at earlier than expected ages, whether arising after irradiation or as a factor unrelated to that form of therapy. In the group of 27 patients for whom neither radiation nor genetic disorder could be implicated, there were 4 with colon carcinoma and glioma and 5 with leukemia or lymphoma and glioma. These combinations may reflect new tissue-specific hereditary cancer syndromes.

Significance to Biomedical Research and the Program of the Institute: The study of children with malignant neoplasms is of significance because: (1) influences of genetic etiologic mechanisms can be more fully appreciated by studying readily available siblings and parents; (2) the possible influence of intrauterine or other etiologic agents is more readily

ascertained because of the short period of possible exposure that limits the number of possible oncogens to be considered; and (3) once cured of cancer, prolonged survival allows for the study of effects of treatment for which long latent periods may be required, such as the induction of SMN by chemotherapeutic agents. The rarity of childhood cancer makes significant studies at single institutions difficult. CICI, as a cooperative group of large pediatric cancer centers with excellent clinical and record keeping facilities, affords the opportunity to study unusual problems of childhood cancer which require larger groups than any single institution could accumulate. Data from this inventory will, for the first time, be able to establish accurate incidence rates of 1) SMN; 2) cancer in sibs; and 3) other associated conditions in patients and family members. It will also provide an inventory of unusual tumor types and make possible comparisons between incidence patterns among geographic groups because of the international scope of the inventory. A body of information related to detailed treatment and subsequent late effects will also be available. The identification of new groups of high risk patients may evolve from this accumulated body of epidemiologic information by discerning new patterns of tumor-tumor or tumor-anomaly associations. The data may also provide an understanding of possible etiologic mechanisms contributing to the neoplastic process.

Proposed Course: (1) Continuation of the CICI with registration of all new cancer patients at the eleven institutions and the development of an appropriate system of data analysis to include follow-up as well. (2) Continuation of the study of non-oncogenic effects of cancer therapy utilizing new methods to assess organ function and possible early impairment such as neuropsychologic tests and radionuclide scans. Surveillance of long-term survivors will continue in order to detect early premalignant organ changes. (3) Continuation of the study of genetic consequences of treatment vis-a-vis specific chromosomal changes and effects on offspring of cured patients. (4) New projects under active consideration include an in-depth review of all 135 patients with SMN with a view toward detecting: a) influence of treatment sequence including radiotherapy and specific chemotherapeutic agents; b) effect of radiation on subsequent SMN with reference to volume, dose and edge effects; and; c) differences in histo-pathology and subsequent response to treatment between SMNs and naturally occurring tumors of the same general type. Examination will also be made of surrounding non-malignant tissues to determine the extent of chronic damage and any indication of premalignant change.

Date Contract Initiated: June 26, 1972

Current Annual Level: $96,276

COLUMBIA UNIVERSITY (NO1-CP-23234)

Title: Analysis of the Mechanism of Chemical Carcinogenesis in Epithelial Cell Cultures

Contractor's Project Director: Dr. I. Bernard Weinstein

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: The overall goal of this contract is the development of epithelial cell systems which will be useful as a rapid assay system for enivronmental agents that may cause cancer and for fundamental studies on the mechanism of chemical carcinogenesis.

Major Findings: 1) An important problem in in vitro carcinogenesis, particularly with epithelial cultures, is the development and validation of specific and objective markers of transformation. It has been found that whereas plasminogen activator (PA) production is a useful marker for the in vitro transformation of fibroblast cultures, it is not a reliable predictor of the tumorigenicity of rat liver epithelial cultures. In a series of 22 normal and tumorigenic epithelial cultures, P.A. production did not correlate with ability to grow in soft agar, morphology, tumorigenicity or metastatic potential. As previously reported, growth in soft agar correlated extremely well with the tumorigenicity of epithelial cultures. Certain cytologic criteria were also found to be reliable predictors of the tumorigenicity of the epithelial cultures. 2) Following the discovery that the phorbol ester tumor promoter (TPA) induces P.A. in cell culture, over 14 related diterpenes have been screened and a good correlation has been found between their ability to induce P.A. and their action as tumor promotors on mouse skin. Certain structure-function requirements have also been elucidated. 3) TPA was found to induce other effects in normal cell cultures that mimic properties characteristic of transformed cells. These include altered morphology, increased saturation density, and changes in the profile of ^{3}H-fucose labelled cell surface glycopeptides. The exposure of transformed cells to TPA further potentiates the expression of the transformed phenotype. 4) Previous findings confirm that TPA enhances the transformation of fibroblast cultures pretreated with an initiating agent. This finding has also been extended to other plant diterpenes. 5) Finding that Dexamethasone can inhibit P.A. production in cell culture suggested that this might explain its ability to inhibit the promotion phase of skin carcinogenesis. A study of a series of potent synthetic glycocorticoids indicated an excellent correlation between these two effects. 6) Further progress has been made in a method for enucleating cells and in other techniques to be used in analyzing nuclear-cytoplasmic interactions during in vitro carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: Although more than 80% of human tumors arise from epithelial tissues, most of the previous tissue culture work related to chemical carcinogenesis (and to oncogenic viruses) has employed fibroblast or embryonic cell cultures. There is a paucity of cloned epithelial cell cultures which can be used for in vitro studies on chemical carcinogenesis. The development of such systems will facilitate studies on the mechanism of chemical carcinogenesis and will also provide rapid and simple bioassays for compounds which may be carcinogenic. Since promoting agents may often be important determinants in human cancer causation, it is essential to develop in vitro systems for the bioassay and study of not only initiating agents but also promoting agents and other cofactors.

Proposed Course: Further studies on the transformation of epithelial cultures will be performed utilizing combinations of chemical carcinogens, promoting

agents and viruses. Preliminary studies suggest that growth in agarose is a more sensitive indicator of transformation than growth in agar and this criterion will be further evaluated with epithelial cultures. The induction of plasminogen activator in cell cultures will be further studied as a potential rapid in vitro assay for promoting agents. In addition to the phorbol esters, other promoting agents and cofactors such as cigarette smoke condensate fractions, anthralin, detergents, various phenols and asbestos will be assayed. Possible interactions between the cellular effects of TPA, synthetic glucocorticoids, vitamin A and various hormones will be examined in epithelial and fibroblast cell cultures.

Date Contract Initiated: April 1, 1972

Current Annual Level: $264,000

ERDA-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY) (YO1-CP-50200)

Title: NCI-ERDA Carcinogenesis Program

Contractor's Project Director: Dr. Theodore T. Odell, Jr.

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The broad objective of the program is to provide an interdisciplinary research approach to solving problems of chemical carcinogenesis. This includes research in respiratory carcinogenesis, tumor cell membrane and DNA repair, in vitro carcinogenesis and metabolism and activation of chemical carcinogens. Considerable progress has been made toward establishing an interdisciplinary approach to the overall problem of chemical carcinogenesis. However, for the purpose of emphasizing the major findings and significance of the various research components, they are presented individually in this report.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,230,091

1. Repair Mechanisms in Carcinogenesis: Membrane Turnover

Specific Project Director: Dr. John S. Cook

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: Transformed cells show enhanced transport of small metabolites, and in some cases the safety factor of transport over growing cell's requirements may be so low as to approach being rate-limiting. An area of concern is how the changes in transport properties are related to the regulation of metabolism in the transformed cell and how changes in transport properties are related to the regulation of growth in both transformed and nontransformed cells. Of special concern are mechanisms and how development of the transformed phenotype represents modification or faulty regulation of normal

physiological processeses such as the induction of transport systems or the turnover of membrane enzymes. Much of the work with cultured human cells has been done with HeLa S3 or a diploid skin fibroblast HSWP. For controllable transformation systems, work has recently been initiated with hamster cell lines derived from BHK. These lines are temperature-sensitive for transformed functions, and have been chemically transformed with nitroso-methylurea, 4-nitroquinoline oxide, or dimethylnitrosoamine. With such cells, growth at the permissive temperature yields a tumorigenic population, whereas growth at the nonpermissive temperature yields a normal population; direct comparative studies can be made on the membrane properties of genetically identical tumor and normal cells.

Major Findings: (1) For induction and turnover studies, HeLa cell membrane proteins have been characterized by their eloctrophoretic properties. More reliable than nonspecific "membrane proteins", the use of 5'-nucleotidase as a quantitative marker has been developed for preparation and analysis of isolated membranes. It has been shown that it is more than 95% localized as an ecto-enzyme on the plasma membrane and that its activity per unit of surface is independent of culture density or position of cells in the cell cycle. The enzyme assay has been optimized and thoroughly characterized as a membrane-bound activity in terms of substrates, inhibitors, metal requirements, pH optima, etc. (2) All environmental agents interact with cells first through the cell membrane. Some attach to specific receptors on the cell surface and are taken up in a "piggyback" mechanism by internalization of the receptor, after which the internalized agent further interacts with the cell via one of a number of possible pathways. Using for a model system the cardiac glycoside ouabain as a surface-specific ligand that interacts with and inactivates a known receptor (Na^+-K^+-ATPase) that is available in radioactive form and whose surface activity can be assayed by assaying receptor activity, an intact-cell analysis for the uptake and detoxification mechanism has been developed. The analysis has been confirmed by cell fractionation studies. (3) The hexose transport system is inducible both by substrate deprivation in normal cells or by cell transformation to malignancy. The two are being compared. In the former case, substrate deprivation of normal human cells leads to enhanced transport capacity by inhibition of the degradative side of turnover of the transporter. It simultaneously leads to an altered metabolism in which phosphofructokinase is inhibited, hexose-monophosphates accumulate and are shunted through the phosphogluconate pathway and lactate excretion falls to a low level. Metabolism and transport changes are independently regulated. Parallel experiments with temperature-sensitive BHK 21 cells undergoing malignant transformation have recently been initiated. Hexose-transport enhancement (< 4-fold in NMU-transformed cells) is an early event in this transformation and is a useful assay in this regard. No information on mechanism is yet available. (4) Normal human cells during growth can concentrate amino acids (α-amino-isobutyric acid, AIB) 25-fold, but in quiescence only 10-fold. It has been shown that this difference is due solely to the membrane potential acting as a force on the Na^+-influx to which AIB uptake is coupled; the ultimate growth-related factor is the transport of alkali cations which is currently being investigated. Similarly, DMN-transformed hamster cells during growth have a 2-fold enhanced amino acid transport capacity when

compared to their normal counterparts. The role and nature of the membrane potential in this system is being investigated as well. With valinomycin-(K^+-ionophore) clamped potentials, the forces on uptake can now be standardized, meaningful kinetic measurements on uptake can be made, and the proposed synthesis of new amino-acid transporters and/or activators on transformation can be assessed.

Significance to Biomedical Research and the Program of the Institute: Current thinking about tumorigenesis emphasizes the altered state of the surface membrane. Such alterations include changing (usually enhanced) activity of transport systems. This program is concerned with: (a) the nature of the enhancement, whether a genetically regulated increase in numbers of transport molecules or an increase in their specific activity; (b) the physiological role of enhanced transport in relation to growth in transformed cells.

Proposed Course: Work in the next year will increasingly emphasize direct comparisons of chemically transformed with nontransformed hamster cells.

The enhanced hexose transport of transformed cells will be investigated for rate-limiting steps, including the probable cellular redistribution of limiting enzymes (hexokinase) from cytosol to membrane-bound forms with altered susceptibility to physiologic regulators. The induction of fructose bisphosphatase in tumor cells and in substrate deprived normal cells, together with its hormonally-dependent regulators, will also be investigated.

The role of the membrane potential in regulation of amino acid transport will be studied in detail. The problem of Na^+-dependence, which has been questioned on quantitative grounds and which has been obscured in eucaryotic cells by an uncertain degree of Na^+ sequestration in the nucleus, will be investigated in cytochalasin B-enucleated cells in which cytoplasmic Na^+ concentration can be determined with reasonable certainty. In such a system, where the transport forces can be established ad-lib, the true cellular capacity for amino acid uptake in the various cell types can be quantitated. The effects of changes in alkali-cation metabolism on transformation, possibly the fundamental event in the enhancement of other transport systems coupled to the membrane potential, will be quantitated so as to account for membrane potentials in transformed, growing but nontransformed, and quiescent cells.

2. DNA Repair Mechanisms in Chemical Carcinogenesis

Specific Project Director: Dr. James D. Regan

Project Officer (NCI): Dr. Rufus Day

Objectives: The major objectives of the DNA Repair Program are to define and characterize the various kinds of prereplication and postreplication DNA repair that occur in human cells after treatment with representative carcinogens and metabolites of carcinogens, in an effort to understand the relationship between the lesions induced in DNA, their alteration by repair enzymes, and the ultimate consequences of these events.

Major Findings: The carcinogenically active form of benzo(a)pyrene (BP) has been postulated to be a BP diol-epoxide. A series of BP epoxides and diol-epoxides were studied for their ability to cause repairable damage to human skin fibroblast DNA. DNA repair can be categorized as ultraviolet (UV) or ionizing radiation type repair using the BrdUrd photolysis technique and following the repair kinetics. As an additional check, repair can be followed in xeroderma pigmentosum (XP) cells known to be deficient in the UV type repair system. Results have shown only the 7,8-diol-9,10-epoxy BP isomers elicit damage to the DNA which is repaired by the UV type repair process. Normal skin fibroblasts are known to be deficient in the enzyme systems thought to be responsible for conversion of the BP precursors to the BP diol epoxides which give UV type repair. Recent findings (PNAS U.S. 74: 238, 1977) show that chronic infection with RLV resulted in rat cells resembling an XP-variant as regards the ability of semi-conservative DNA replication to cope with 4-nitroquinoline oxide-induced lesions in its DNA. The defect in the variant is responsible for the XP phenotype and an increased frequency of induced mutation. Further studies with RLV-infected cells may clarify the molecular mechanisms of virus-induced carcinogenesis or cocarcinogenesis and its relationship to mutagenesis (induced by environmental chemical carcinogens) and DNA repair.

Significance to Biomedical Research and the Program of the Institute: In all experiments involving the treatment of cells or organisms with chemical carcinogens, repair of the lesions induced by the agents is a significant chemical event and should be investigated. It is conceivable that carcinogenesis may occur as a result of residual, i.e., unrepaired lesions, in the DNA. In this institute, extensive experiments involving treatment of cells, animals, and microorganisms with chemical carcinogens are being carried out. Thus it is most essential that there be, as an integrated part of this program, a laboratory working exclusively on DNA repair after treatment with chemical carcinogens.

The significance of these studies lies in: 1) The ubiquitousness of repair (most organisms including man have several complex repair systems); 2) the belief that mutagens and carcinogenic events may arise only from residual (nonrepaired) lesions or that error-prone repair systems may be the major induction mechanisms of the mutagenic or carcinogenic event; 3) the clear association of repair defects and highly carcinogenic disease states in man (Xeroderma pigmentosum).

Proposed Course: To continue these experiments on prereplication repair using selected carcinogens and their metabolites and to determine in molecular terms the repair mechanisms already found operative with certain carcinogen metabolites. In postreplication repair, to determine if the relationship found between Rauscher leukemia virus and a defect in post-replication repair is a valid one for other virus-cell-carcinogen systems.

3. Mechanism of Action of Chemical Carcinogens

Specific Project Director: Dr. James K. Selkirk

Project Officer (NCI): Dr. Andrew Peacock

Objectives: To develop in vitro methods for utilization of homogenous cell types more closely related to human carcinogenesis and to investigate the metabolism of chemical carcinogens in terms of their metabolic profile, their kinetics of activation and detoxification, isolation and identification of active carcinogenic intermediates and elucidation of the differences between carcinogenic and noncarcinogenic isomers of the same polyaromatic molecule. This includes variation in the kinetics of metabolism, emphasizing: 1) formation and half-life of the active carcinogenic species; 2) quantitation of binding to cellular macromolecules; 3) development of innovative analytical methods to isolate and identify the activated macromolecular species and detoxification products of several types of chemical carcinogens.

Major Findings: Several rodent cell types, some susceptible and some resistant to in vitro malignant transformation, have been studied to find out if there are qualitative variations in the metabolites produced and to estimate the inducibility of the mono-oxygenase metabolizing enzymes.

The absolute yield of the various hydroxylated metabolites is dependent upon the type of incubation employed. The relative yield of dihydrodiols and phenols is different for hepatic microsomes, lysed cells in culture, and long-term incubations with intact cells. Whereas the predominant metabolite for hepatic microsomes is the 3-hydroxy-benzo(a)pyrene, the 9-hydroxy-benzo-(a)pyrene becomes the major metabolite in quasi-microsomal preparations where cells are broken and the incubation conditions are identical to using hepatic microsomes. However, the relative metabolite yield from the metabolism of benzo(a)pyrene in intact cells in culture is markedly different from the yield when either microsomal or lysed cell preparations are used. Either phenol production is greatly depressed or the phenol products are rapidly metabolized to water-soluble conjugates. The K-region BP-4,5-diol present in hepatic microsomal incubations is absent in the cell incubation. These results found in rodent cells are almost identical to those obtained from bronchial organ culture.

Significance to Biomedical Research and the Program of the Institute: Understanding the metabolism kinetics of activation and detoxification, including the rate-limiting steps of formation and removal of the active carcinogenic species, will be critical to understanding the role of these compounds in cancer in man.

Proposed Course: Using high-pressure liquid chromatography and various types of fibroblast and epithelial cell lines, both susceptible and resistant to in vitro transformation by polycyclic hydrocarbons, the mode of action of these powerful carcinogenic environmental pollutants can be determined. Close collaboration with several groups internally at the Oak Ridge National Laboratory and at the National Cancer Institute for the selection, assay, and understanding of the metabolic activation mechanism for polycyclic hydrocarbon carcinogens has led to the hope of extracting from this data base pertinent information on carcinogenic interaction with target tissues. Collaborative studies have been undertaken to follow long-term benzo(a)pyrene activation in metabolism in mouse skin epidermal cells which are primary cells and are presumed as the actual target-site cells for polyaromatic carcinogens. Studies on RNA metabolism in susceptible, resistant and

premalignant cells have begun in collaboration with another group in the Biology Division. These studies are designed to determine the effect of formation of modified bases by polynuclear aromatic hydrocarbons. It is anticipated that the information derived from this collaborative effort will directly benefit these studies.

4. Chemical Carcinogenesis Cell Biology

Specific Project Director: Dr. Raymond W Tennant

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: The objective of this project is to independently evaluate the transplacental assay system developed by DiPaolo and co-workers (1973) for identifying the oncogenic potential of chemicals.

Major Findings: A protocol, based on colony morphology, was established and used in 1975-76 to test chemicals of known and unknown carcinogenic activity in the host-mediated in vivo-in vitro assay. However, in 1976-77, the development of problems in cell culture, primarily related to the health status of test animals, has required a change in the criteria used to assess transformation. Progress has been made in applying the focus assay method. Continued development of this technique in the host-mediated assay system may lead to a more defined and reliable estimate for determining the oncogenic potential of chemicals.

Hamsters from both commercial and private colonies have been tested for health status and other factors in the host-mediated assay. Currently, animals obtained from Engle Farm Laboratory provide the most reliable source. Considerable effort was also directed at testing various culture media used in performing the colony assay. Dulbecco's modified medium marketed by KC Biological has provided the best growth support for hamster cells if supplemented with 10% unheated Reheis fetal calf serum (FCS). A cloning efficiency of 25% has been routinely obtained without any feeder layer; however, the cloning efficiency of the hamster cells was reduced by more than 50% if Reheis FCS was heat-inactivated at 56° for 30 minutes.

The scoring of morphologically transformed colonies requires some subjectivity in appraisal. Many colonies must be examined for transformation, especially when the transformation frequency is not high. In addition, a battery of in vitro criteria have been tested in this laboratory to aid in scoring transformed colonies, such as the differences in staining of colonies related to the nucleus/cytoplasm ratio, peripheral cell orientation, colony growth rate and density. However, many variables, such as the health status of the dam and culture conditions, strongly influence these criteria independent of carcinogen treatment.

The direct focus assay developed by Casto and co-workers (1976) appears to be influenced by external variables and may be a reliable method for identifying transformed colonies of cells. Chemicals including MNNG, DMBA, benzo(a)pyrene, 3-methylcholanthrene, and beta-propiolactone have induced foci. MNNG has been the most potent carcinogen tested, with trnasformation

frequencies ranging from 200, 13.4, 5.3, to 1.5 per 10^5 survivors at MNNG concentrations of 1.5, 1, 0.5, and 0.1 μg/ml, respectively. The focus induction frequency ranges from 0.76 per 10^5 survivors in DMBA-treated cells to 200 per 10^5 survivors in MNNG-treated cells; it appears to be independent of the cell number inoculated but dependent on chemical concentration and the length of carcinogen treatment. Recent experiments have indicated that the exposure of carcinogen-treated cultures to 5% FCS about two weeks after treatment led to a 2-3 fold increase in transformation frequency as compared with cultures maintained in 10% FCS. Therefore, the expression of transformation in this system appears to be controlled by the serum-regulated cell density of the normal background cells in the cultures.

Significance to Biomedical Research and the Program of the Institute: The host-mediated transplacental or in vivo-in vitro assay can produce semi-quantitative data with no spontaneous transformation frequency and can be used to test potential carcinogenic chemicals including those which need metabolic activation or are solubilized only in organic solvent. This system can also provide a model for host-carcinogen interactions and cancer cell biology.

Proposed Course: (1) To continue the improvement of the focus assay until the methodology is satisfactorily improved for detecting a low frequency of transformation; (2) to improve the methods of scoring for transformation in addition to the focus assay by the application of the fibrin overlay or soft agar technique; (3) to incorporate into the focus assay system a promoter which might augment the effect of subcarcinogenic doses of carcinogens; (4) to study the possibility that tissues other than fibroblasts can be used to identify carcinogens in the transplacental assay.

5. Respiratory Carcinogenesis

Specific Project Director: Dr. Paul Nettesheim

Project Officers (NCI): Dr. Michael B. Sporn
Dr. Carl E. Smith

Objectives: To analyze cocarcinogenic effects of physical and chemical agents in the induction of lung cancer; to identify and study those environmental agents which may increase the susceptibility of respiratory tissues to normally ineffective doses of carcinogen or which may alter the "effective" tumorigenic dose by affecting distribution, deposition, and/or clearance of inhaled carcinogenic particulates; to study host factors that affect tumor induction and tumor progression; to investigate the inhibitory effects of retinoids on the development of respiratory tract neoplasia; to identify the major steps in the evolution of respiratory tract cancer using in vivo and in vitro approaches and to uncover early indicators (markers) for preneoplasia to develop new experimental models for cofactor studies.

Major Findings: (1) Evaluation is being made of an inhalation study which was conducted to determine whether reactive gases produced during combustion of fossil fuels, namely nitrogen dioxide, formaldehyde, and acrolein, enhance the carcinogenic potency of benzo(a)pyrene given by intratracheal injection. The time relationship between carcinogen administration and irritant exposure

was investigated. Data on seven different NO_2-carcinogen exposure groups (80 hamsters per group) show that NO_2 does not cause any measurable enhancement of pulmonary carcinogenesis induced by BaP. Statistical evaluation of similar studies with formaldehyde and acrolein are underway.

(2) A new series of studies was initiated to examine the effects of two retinoids on the development of respiratory tract neoplasia in hamsters. Feeding of relatively nontoxic retinoids encapsulated in gelatin beadlets makes it possible to obtain high and sustained serum titers. The first study showed that at one level of carcinogen exposure (MNU) the highest nontoxic retinoid dose reduced the respiratory tumor incidence by 25-35%. Further studies with different carcinogen exposures and different levels of retinoid intake are currently underway and indicate larger effects at lower carcinogen doses.

(3) An in vitro system for tracheal organ culture previously developed was used to study initiation and promotion in respiratory tract epithelium. Results obtained to date have shown that respiratory tract epithelium can be transformed in vitro using chemical carcinogens. They also showed that the promoter TPA stimulates DNA synthesis in this epithelium in a dose-dependent fashion. Promotion experiments are currently in progress. Studies on epithelial cells initiated in vivo show that such cells acquire an unlimited growth capacity in vitro soon after a brief carcinogen exposure. With continued in vitro culture, many of these cell cultures become neoplastic. Thus, the progression of epithelial cells from an initiated preneoplastic to a neoplastic state can be studied in vitro.

(4) The tracheal transplant system was developed to investigate the role of cofactors (environmental and host factors) in respiratory tract carcinogenesis and to study the evolution of epithelial neoplasia in a preselected site of the respiratory tract. Dose-response studies with the tracheal transplant system have shown that the minimum tumorigenic dose for benzo(a)pyrene is 300 µg and for dimethylbenzanthracene 30 µg. Based on this dose-response information, several studies were initiated to study the cocarcinogenicity of asbestos and of benzo(a)pyrene. Studies on the age specific susceptibility of tracheal epithelium are also underway. Based on preliminary experiments, a large study was initiated to investigate the fate of various epithelial lesions induced by a brief exposure to DMBA which will induce between 20-40% carcinomas in 18-24 months. This study involves approximately 300 tracheal transplants which are sampled at 2-4 month intervals. The initial findings are confirmed showing that the early generalized hyperplastic-metaplastic response gives way to atrophic and normal epithelium within a few weeks. Subsequently focal metaplastic lesions develop which show no cellular atypia (2 months). At 4 months, the majority of metaplasias have various degrees of cellular atypia. Dysplasias characterized by almost complete absence of squamous differentiation appear together with metaplasias with severe atypias. Final results are expected in 9-12 months.

(5) The tracheal transplant system has been effectively utilized as a source of large quantities of mucus which has permitted the biochemical study of the glycoprotein moieties in this material. Isolation of the glycoprotein fractions has been carried out and these fractions are currently being

analyzed for their amino acid and sugar content. This approach should prove to be extremely useful in the study of the changes in the biochemical and biophysical properties of mucins after exposure of the tracheal transplants to various toxic agents.

An in vitro tracheal organ culture has been developed in which the control of cell proliferation and cell differentiation in respiratory tract epithelium can be readily studied. Culture conditions which favor development of either secretory cells, undifferentiated cells, or keratin producing cells have been determined. Presently the effects of vitamin A on the development of secretory cells and the synthesis and turnover of the mucin glycoprotein by these cells is in progress.

(6) Another localized tumor induction system was developed a couple of years ago. Repeated washings of a circumscribed region of the hamster trachea with NMU are performed using an automatic syringe designed to collect exfoliative cytology. However, no dose-response data are available. Therefore, studies were undertaken to evaluate the following exposure parameters: carcinogen concentration, number of exposures required to effect approximately 30, 50, and 90% tumor incidence, length of interval between exposures. The optimum exposure frequency and time interval between exposures to 1% NMU to induce the desired tumor incidence with minimal acute mortality has been obtained. Studies exploring the effects of changing the NMU concentration are in progress. This tumor induction system is anticipated to be of considerable value in studying the progression of neoplasia and factors affecting the postinitiation phase of respiratory tract tumor development.

Significance to Biomedical Research and the Program of the Institute: Epidemiologic data indicate a multifactorial etiology in human lung cancer. This work is a study, using animal models, of the role of inhaled chemicals and of physical and biological agents influencing the respiratory tract in the development of lung cancer. It is expected that this approach will help to define the decisive environmental and host factors in the pathogenesis of lung cancer. The studies are also aimed at elucidation of the evolution of lung cancer, particularly the early preneoplastic phases, in order to identify the essential steps in the development of this neoplastic disease.

Proposed Course: (1) To develop better systems than are currently available to test cocarcinogenic effects of toxic enhalants. To finalize the evolution of the cocarcinogenesis studies with formaldehyde and acrolein.

(2) To evaluate the anticarcinogenic effects of synthetic retinoids using a localized lung tumor induction system in hamsters.

(3) To study neoplastic transformation of respiratory tract epithelium in organ and cell cultures and to develop criteria for transformation of such cells. To study two stage carcinogenesis in vitro using respiratory tract epithelium.

(4) To develop indicators and markers for preneoplasia and early neoplasia for respiratory tract epithelium.

(5) To conduct cocarcinogenesis studies with the tracheal transplant system.

HARLAN INDUSTRIES (NO1-CP-55647)

Title: Development of Colonies of Aged Rats

Contractor's Project Director: Mr. Hal P. Harlan

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this contract is to breed the inbred strain (obtained from the Alton Ochsner Medical Foundation) and Sprague-Dawley derived outbred stock rats (obtained from the Veterinary Resources Branch, DRS, NIH) for the Carcinogenesis Program. Males of both are placed in a barrier facility for aging purposes: one-half of the males of each will remain virgins, the remainder will be exposed to maximum sexual activity.

Major Findings: The project is progressing well, and we are now into the third phase of the project and are aging both the Sprague-Dawley stock and the ACI strain of rats in our barrier colonies. The oldest animals inhouse are 15 months of age, with survival in excess of 95%.

Significance to Biomedical Research and the Program of the Institute: During the latter period of live in both man and animal, a number of physiologic alterations occur which may be significant events in the carcinogenesis process. One of the cancers thought to be a manifestation of age is prostatic cancer, as judged by experimental evidence developed in the Carcinogenesis Program over the past three years. These animals will be available, at various ages, to permit experimental verification and analysis of this phenomenon.

Proposed Course: To maintain these animals, to provide pertinent information to the Program, such as survival rates, health status and colony sizes, and to distribute these animals as appropriate to various investigators through the Carcinogenesis Program.

Date Contract Initiated: June 30, 1975

Current Annual Level: $213,816

IIT RESEARCH INSTITUTE (NO1-CP-65745/BOA)

Title: Preparation of Carcinogens

Contractor's Project Director: Dr. Allan P. Gray

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To supply quantities of well-characterized chemical carcinogens, of consistent, reproducible and documented composition and purity to the Chemical Repository for distribution for carcinogenesis research; to provide complete information as to synthetic procedures or compound sources, physical properties, purity, analytical data; to carry out stability studies on certain of the carcinogens and furnish information as to optimum storage conditions and rates of degradation.

Major Findings: To date, the following compounds have been synthesized, or purchased and purified, and supplied to the Repository: benzo(e)pyrene, glycidaldehyde, N-2-fluorenylacetamide, butyl-4-hydroxybutylnitrosamine (additional quantities are in process), nitrosomethylurea, 4-hydroxyl-amino-quinoline N-oxide, N-nitroso-N-ethylaniline. Synthesis of the following compounds is expected to be completed in the near future: bis-(chloromethyl) ether, N-acetoxy-2-fluorenylacetamide.

Significance to Biomedical Research and the Program of the Institute: The need has been recognized for the Carcinogenesis Research Program to be able to supply researchers in carcinogenesis with well-characterized samples of carcinogens for their investigation. Samples of compounds under study should be of consistent, reproducible, and documented composition and at as high a level of purity as feasible in order that investigators in different laboratories, or in the same laboratory at different times, can be confident of the nature of the material they are working with and of the validity of comparative data. One possible source of variability in research results can thus be eliminated.

Not only has it been difficult to obtain carcinogens of consistent composition from the usual commercial suppliers, but many suppliers have elected to stop handling suspected carcinogens. Thus, many of the compounds desired for research are no longer available commercially at all. Our work is carried out in a toxic laboratory facility designed to meet OSHA requirements for handling carcinogens. Valuable experience is thereby gained in the safe handling of bulk quantities of these materials.

Proposed Course: This project will continue to synthesize and supply compounds as requested by NCI for distribution by the Chemical Repository.

Date Contract Initiated: November 1, 1975

Current Annual Level: Since this is a Basic Ordering Agreement, the level of expenditure is assigned to each task as it is developed.

IIT RESEARCH INSTITUTE (NO1-CP-55646)

Title: Chemical Repository

Contractor's Project Director: Dr. James N. Keith

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To establish a facility for the safe storage, repackaging, and distribution of potential chemical carcinogen reference samples for use in cancer research.

Major Findings: Stocks were transferred to the remodelled facility and procedures established early in this fiscal year. Since initiation of the program, the inventory has grown from 74 to 168 compounds, 50 of which are hydrocarbon metabolites and analogs. The program is supplying coded samples to 14 contractors participating in a mutagenicity-carcinogenicity double-blind study for the In Vitro Program of the Bioassay Operational Unit. A total of over one thousand individual samples of carcinogens and reference compounds have been shipped, and numerous requests for general and specific information on handling chemical carcinogens have been answered.

Stock for the Chemical Repository is purchased from commercial suppliers and reanalyzed for purity or received from one of the NCI contractors responsible for synthesis of these compounds. Samples of surplus bioassay stock are also being sent to the Repository for storage.

Significance to Biomedical Research and the Program of the Institute: Besides the need for effective coordination of information flow, the carcinogenesis research community has a pressing need for a centralized source of well-documented reference compounds. To this end, a chemical repository for the safe storage, repackaging, and distribution of samples for the Carcinogenesis Program has been established. This facility, designed and operated in conformance with OSHA and EPA regulations, receives material from suppliers, repackages as required for the users, and ships samples with analytical documentation and safe handling protocols.

Proposed Course: The Repository is becoming a useful distribution center for chemical carcinogens no longer available on the market and performs a number of other service functions as well. Support activities will continue throughout the five-year contract.

Date Contract Initiated: June 29, 1975

Current Annual Level: $200,000

IIT RESEARCH INSTITUTE (NO1-CP-33296)

Title: Production and Characterization of Particulate Materials for Respiratory Studies

Contractor's Project Director: Ms. Jean Graf

Project Officer (NCI): Dr. David G. Longfellow

Objectives: (1) To prepare and characterize powder fractions needed for lung retention studies; (2) to prepare and characterize powder fractions coated with any polynuclear hydrocarbon carcinogen of interest to the National Cancer Institute; and (3) to prepare and characterize dispersions

of coated and uncoated particles in any vehicle or solvent of interest to the National Cancer Institute.

Major Findings: Size fractions of carbon and the oxides of iron, aluminum, nickel, and cobalt have been prepared and characterized. These have been successfully coated with benzo(a)pyrene (BP) and maintained in a stable form for periods up to three months. Suspensions of BP in saline and gelatin have been prepared and characterized. The preparations have been distributed to institutions currently performing studies under the NCI repository carcinogenesis program and are available to other interested researchers upon request.

Individual manufacturers' lots of BP have been found to behave differently when coated on iron oxide. Particle size and degree of attachment of BP to iron oxide in the coated product are the properties which vary when different BP lots are used. Ames' bioassay studies of six different manufacturers' lots of BP have indicated that all lots are mutagenic.

Degradation of BP both during the preparation and the storage of coated oxide powders has been an area of concern. While one group of studies on a separate contract indicated no BP degradation during these processes, further questions raised by NCI personnel have led to the study of BP-coated iron oxide powders by a high-pressure liquid chromatographic (HPLC) technique. The approach has detected the presence of BP degradation products in coated iron oxide samples, especially after long storage periods following exposure to ultraviolet light. This finding is significant in that the HPLC technique detected phases undetectable by gas chromatographic techniques.

Significance to Biomedical Research and the Program of the Institute: A constant, well-characterized and dependable source of particulate materials for intratracheal instillation experiments is available for use by the carcinogenesis research community.

Proposed Course: To continue this support service to NCI through FY 77. Remaining stock at that time will be transferred to the Chemical Repository for distribution.

Date Contract Initiated: June 10, 1971

Current Annual Level: $92,500

MEDIZINISCHE HOCHSCHULE HANNOVER (NO1-CP-12148)

Title: Development of a Lung Tumor Model with Large Wild European Hamsters (*Cricetus cricetus* L.)

Contractor's Project Director: Dr. Ulrich Mohr

Project Officer (NCI): Dr. Carl E. Smith

Objectives: This contract was initiated to explore the possible usefulness of the European hamster (*Cricetus cricetus* L.) as an animal model for the induction of respiratory tract tumors morphologically similar to tumors found in man. The specific aspects are to (1) capture the wild hamsters and establish a breeding colony, (2) determine the incidence of spontaneous tumors, especially bronchogenic carcinomas, (3) test for sensitivity to induction of lung cancer by known carcinogens, and (4) attempt to develop other animal model uses for this animal.

Major Findings: While feral animals continue to be captured and utilized in some experiments, the development of a domestic colony continues with excellent success. The domestic colony now numbers almost 600 animals and is in the F_8 generation. Preliminary efforts are being made on a small scale to develop a melanistric line; this is now in the F_2 generation, however, only normally coloured animals have been born. A breeding colony has been established at NIH and animals are available in limited quantities for experimentation. The nitroso-compounds study in the domestic animals has been continued and expanded. Evidence to date confirms that these animals are indeed sensitive to the carcinogenic effect; exposure results in high incidences of bronchogenic carcinoma and tumors of the nasal and oral cavities with a specific nitroso compound dependency.

An English language anatomical atlas of the European hamster has been prepared and is now in press. Efforts are being made to determine if the methods used to produce pancreatic tumors in the Syrian hamster will result in comparable results in the European hamster.

Significance to Biomedical Research and the Program of the Institute: Regardless of the causative factors of bronchogenic carcinoma, the availability of laboratory animals with this characteristic would be invaluable to cancer research. Other characteristics which would make this animal superior to the Syrian golden hamster are its larger size and its longer lifespan. At present there is no satisfactory animal model for the study of the pathogenic squamous cell carcinoma. Moreover, preliminary trials have shown that the larger size of the European hamster permits the direct injection of carcinogens, e.g., locally acting B(a)P into the main bronchus under sight control of a specially adapted bronchoscope. Bronchoscopy is then used as a monitoring procedure to observe the development of so induced bronchogenic tumors.

The program is of direct and intimate relevance to the lung cancer program. If this species can be bred and maintained as a laboratory animal, it would be a valuable animal for the bioassay program, as well as to study mechanisms and pathogenesis of lung cancer.

Proposed Course: Initially it was necessary to capture and adapt this wild species to laboratory conditions and to develop a breeding program. The natural occurrence and incidence of diseases, especially lung tumors, has to be determined as this animal has a relatively longer lifespan than the commonly used laboratory rodents.

At present, the chronic testing of known carcinogens by various routes, as well as studies using intratracheal B(a)P instillation and cigarette smoke inhalation are being conducted.

In addition, investigations using bronchoscopic procedures are now being further expanded to permit the observation and recording of the developmental course of induced lung tumors.

Efforts are also being continued to develop the European hamster as a valid animal model for pancreatic carcinogenesis.

Date Contract Initiated: June 22, 1971

Current Annual Level: $80,000 ($20,000 IRP&R; $60,000 Lung)

MIDWEST RESEARCH INSTITUTE (NO1-CP-65739/BOA)

Title: Preparation of Carcinogens

Contractor's Project Director: Mr. James C. Wiley, Jr.

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To provide adequate quantities of high purity and well-characterized reference compounds to the Chemical Repository for distribution to the carcinogenesis research community. The project is designed to serve a dual purpose: to synthesize compounds that are unavailable from other sources; and to provide analyzed chemicals to researchers to eliminate variability in experimental results.

Major Findings: The carcinogen 7-bromomethyl-12-methylbenz(a)anthracene was prepared and shipped to the NCI Repository. Progress has been made on the development of a new synthetic procedure for the preparation of 7,9-dimethyl-benz(c)acridine.

Significance to Biomedical Research and the Program of the Institute: This contract will provide significant quantities of well-characterized reference compounds for carcinogenesis research to enable investigators to identify and study the mechanisms of action of carcinogenic substances and their metabolites.

Proposed Course: The project will continue to provide quantities of well-characterized carcinogens as requested by NCI.

Date Contract Initiated: January 21, 1976

Current Annual Level: Since this is a Basic Ordering Agreement, the level of expenditure is assigned to each task as it is developed.

MIDWEST RESEARCH INSTITUTE (NO1-CP-23270)

Title: Analytical Chemistry Resource

Contractor's Project Director: Dr. Evelyn Murrill

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The objective of this program is to serve as a state-of-the-art analytical facility for the Carcinogenesis Program. Its purpose is to precisely define the chemical nature of compounds to increase the accuracy and reliability of data obtained in carcinogenesis research. When the program began in June 1972, the scope of tasks included analysis of chemicals, chemical/feed mixtures and feeds for the Bioassay Operational Unit as well as specialized analysis for investigators in other units. In July 1974, the program was divided into a general analytical resource (this program) and a resource specialized for the Bioassay Operational Unit.

Major Findings: Since June 1972, studies have been completed and reported on 242 batches of chemicals (184 different chemical substances). Ten have been analyzed in detail to identify and quantitate minor impurities, and 30 chemical mixes have been analyzed for stability or homogeneity. Routine characterization studies include chromatography (TLC, GC, HPLC, etc.) elemental analysis, water analysis, and other assay methods dependent on the chemical (titration, voltammetry, mass spectrometry). Physical data include transition data (melting point and boiling point), refractive index and optical rotation, and spectral data (infrared, ultraviolet, visible, mass and NMR). Specialized tasks have included synthesis, identity and assay of pure benzo(a)pyrene; purification and assay studies of dimethylbenzanthracene; and a literature survey on the analytical methodology of the OSHA Carcinogens.

A major portion of the effort during the last six months has been directed to the assay of 3-methyl cholanthrene metabolic products by high-pressure liquid chromatography, mass spectrometry and other analytical methods.

Significance to Biomedical Research and the Program of the Institute: The literature of carcinogenesis studies contains many examples of experiments which cannot be meaningfully interpreted because the test substances had not been adequately characterized chemically. When such an experiment results in low tumor incidence, for example, the question will always remain as to whether the tumors observed might have arisen from small concentrations of highly potent impurities, rather than from the allegedly weak nominal ingredient being tested. This contract provides the analytical expertise and instrumentation necessary to answer questions of identity or purity of chemicals used by researchers in carcinogenesis as well as provide analytical backup to assist the researcher in more sophisticated analytical investigations involving metabolic product assay and identification.

Proposed Course: Continue to provide analytical services as requested by NCI.

Date Contract Initiated: June 28, 1972

Current Annual Level: $24,449

MIDWEST RESEARCH INSTITUTE (NO1-CP-33387)

Title: Synthesis of Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Mr. James F. Engel

Project Officer (NCI): Dr. Shen K. Yang

Objectives: The overall objective of this program is the preparation of NCI-selected polycyclic aromatic hydrocarbon metabolites by unequivocal methods to produce quantities of high purity and well-characterized materials. These compounds are provided to the carcinogenesis research community through the Chemical Repository for use as standards. Activities in support of the NCI Repository include supply of known and/or suspect metabolites, resyntheses to insure a continuing supply, and operation of a repository for isotopically-labelled derivatives (^{3}H, ^{14}C, and ^{13}C).

Major Findings: To date, approximately 90 derivatives of the following five parent hydrocarbons have been prepared: benzo(a)pyrene, 3-methylcholanthrene, benz(a)anthracene, 7,12-dimethylbenz(a)anthracene, and dibenz(a,h)anthracene. The products prepared thus far have included K-region and non-K-region phenols, quinones, epoxides, dihydrodiols, dihydrodiol-oxides, alkyl- and hydroxyalkyl-substituted derivatives, optical isomers, and ^{3}H-, ^{14}C-, and ^{13}C-labelled analogs.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the relationship between the formation of specific metabolites of polycyclic aromatic hydrocarbons and the induction of cancer necessitates the availability of authentic samples of probable metabolites. It would be virtually impossible for a laboratory lacking specific synthetic experience to synthesize any one of these compounds for a specific experiment. This contract permits the Carcinogenesis Program to make compounds available for pertinint experiments in chemical carcinogenesis by all interested laboratories.

Proposed Course: Compounds will continue to be synthesized to meet the scientific needs of the carcinogenesis research community. A major effort will be devoted to the resynthesis of compounds for distribution via the NCI Chemical Repository. These will include K-region and non-K-region derivatives with emphasis on the non-K-region dihydrodiol and dihydrodiol-oxide derivatives of benzo(a)pyrene. In addition, the syntheses of similar derivatives of other parent hydrocarbons will continue, and it is expected that a concerted effort will be directed toward the preparation of ^{14}C- and ^{3}H-labelled derivatives of high specific activity.

Date Contract Initiated: June 15, 1973

Current Annual Level: $384,900

MIDWEST RESEARCH INSTITUTE (NO1-CP-23270/SUB-CONTRACT)

Title: Development of a Selective Method for the Trace Analysis of Hydroxamic Acids

Contractor's Project Director: Dr. Thornton C. Carpenter

Project Officers (NCI): Dr. David G. Longfellow
Dr. Elizabeth K. Weisburger

Objectives: The objective of this program is to develop a selective method for the trace analysis of hydroxamic acids, particularly for use with urine samples.

Major Findings: Iron(III) ions immobilized on Chelex chelating resin provide an effective column chromatographic method for isolating and concentrating N-phenylacetohydroxamic acid from 25 ml of normal human urine to which hydroxamic acid had been added at a level of 1 ppm. No prior clean-up of the urine is necessary. After washing the column, the iron and hydroxamic acid are eluted from the column with dilute hydrochloric acid. The eluate is buffered to pH 5, the iron(III) is sequestered with EDTA, and the free hydroxamic acid is extracted into methylene chloride. Evaporation of the methylene chloride leaves the hydroxamic acid in an almost pure state, which then can be quantitatively assayed by screening methods (spectrophotometrically with reconstituted iron(III) complex) or by more sensitive analytical methods (e.g., HPLC). The hydroxamic acid can be recovered quantitatively from the column eluate, and from the entire chromatographic procedure with work-up at ca 70%, with good precision.

Significance to Biomedical Research and the Program of the Institute: From what is now known about the metabolism of aromatic amines, amides, and nitro compounds compounds, and how such metabolic transformations might relate to carcinogenesis, it becomes apparent that all such compounds should be carefully evaluated as potential carcinogens. Specifically, such compounds should be investigated for the production in vivo of hydroxylamines and hydroxamic acids. It has been concluded that N-hydroxylation is a necessary biochemical reaction to elicit carcinogenic properties in aromatic amines or amides. Those compounds found to be metabolized in such a manner would merit careful evaluation of their safety. It is quite probable that many aromatic amines, amides, and nitro compounds are converted to hydroxylamines or hydroxamic acids in very low quantities, making the detection of such metabolites extremely difficult. Nevertheless, trace production of hydroxylamines and hydroxamic acids may be important to the production of human malignancies. Presently, no rapid and sensitive determination exists that will specifically detect hydroxamic acids in highly dilute solutions.

Proposed Course: This method will be further refined in attempts to lower the minimum detection limits of the hydroxamic acid, and it will be extended to other hydroxamic acids, specifically N-2-fluorenylacetohydroxamic acid. Its applicability to rat urine analysis will also be studied for the purpose of using it in conjunction with bioassay feeding experiments.

Date Contract Initiated: June 30, 1976

Current Annual Level: $25,551

NCI FREDERICK CANCER RESEARCH CENTER (LITTON BIONETICS, INC.) (NO1-CO-25423)

Title: Operation and Maintenance of the Frederick Cancer Research Center

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Carcinogenesis Coordinator (NCI): Dr. Elizabeth K. Weisburger

Objectives: Besides the diverse program of research at the Frederick Cancer Research Center (FCRC) in collaboration with the various divisions of the National Cancer Institute, FCRC also serves as a resource. Approximately 27% of the activities at the Center are supported by the Carcinogenesis Program. The FCRC Chemical Carcinogenesis Program has as a primary objective the development of a unique national resource capable of initiating and evaluating a broad range of approaches to effectively identify human carcinogens. To do this requires a coordinated interdisciplinary approach which consciously emphasizes the integration of basic and applied research. Broadly, the areas of research being supported are:

1. Development of methods and conduct of testing to identify environmental chemicals which are carcinogenic or cocarcinogenic.

2. Investigation of host-chemical interactions with the aim of being able to alter host susceptibility and response after exposure.

3. Attempts to elucidate mechanisms of carcinogenesis on the premise that an understanding of the process will permit the manipulation of factors which will block or reverse tumor formation.

Building a staff and research program into an excellent national resource is necessarily an evolutionary process which requires considerable time. As a result of the cooperative efforts of NCI staff, consultants, and the contractor's staff at FCRC, the Chemical Carcinogenesis Program has initiated research programs in the following areas: (1) Bioassay, (2) In Vitro Carcinogenesis, (3) Synthesis and Analysis of Chemicals for Carcinogenicity Studies, (4) Procedures for the Safe Handling of Chemical Carcinogens, (5) Endogenous Formation of Carcinogens, (6) Endocrine Carcinogenesis, (7) Molecular Aspects of Chemical Carcinogenesis, (8) Cofactors in Chemical Carcinogenesis, and (9) Mechansims of Nitrosamine Carcinogenesis. These projects address various facets of the three general areas of identification of chemicals, chemical-host interactions and mechanism studies. In addition, the areas of synthesis and analysis as well as safety are essential support components for the program at FCRC and for the overall NCI Chemical Carcinogenesis Program. In keeping with the concept of providing a national resource, the program will change its emphasis as necessary to respond to changing national priorities. The interrelationships established are such that changes can be made in a reasonable time with minimal disruption.

In order to provide a better understanding of the program as it is presently evolving and as it is proposed for continuation, each project is presented below in terms of objectives, rationale, examples of results and proposed areas for continuation. Presenting them as discreet projects is simply for the purpose of highlighting the research and is not intended to imply separate and indepentent activity or a rigid organizational scheme. Rather, there is

increasing interaction among programs and it is the intent of the Chemical Carcinogenesis Program to foster in-house collaboration and to extend it to include increasing collaboration between FCRC scientists and those in other institutions.

Project 7

Title: Endogenous Formation of Carcinogens

Contractor's Project Manager (Acting): Dr. Morris Kelsey

Project Monitor (NCI): Dr. Elizabeth K. Weisburger

Objectives: Identification and investigation of the role of carcinogens or promoters formed by the metabolism of endogenous substrates is the primary goal of this research program, with efforts being concentrated on formation in situ in the large intestine. Emphasis is placed on the role that microsomal, microfloral and mucosal enzyme systems may play in the conversion process. Included are investigations of the formation of carcinogens from exogenous substrates which produce carcinogens after entry into the body as well as research on the formation of carcinogens from normal endogenous substrates such as bile acids, cholesterol, and amino acids. The role of endogenous compounds such as bile acids and sterols in the activation or detoxification of known chemical carcinogens is also a major goal of this program.

Major Findings: Studies concerned with the mechanism of the bile acid activation of chemical carcinogens have shown that lithocholic acid (LA) affects the metabolism of 2-anthramine (2-AA) and benzo(a)pyrene (B[a]P). Using B[a]P as a model carcinogen, LA influences the formation of mutagenic metabolites when liver S-9 fractions from phenobarbital-stimulated animals are used to metabolize the carcinogen. If Aroclor-stimulated S-9 fractions are used, a decrease in the number of histidine revertants is observed. 4-[14C]-Cholesterol-α-epoxide administered orally to C3/B6/F1 mice is excreted in the feces mainly as the triol. The majority of the remaining label was found in urine, blood, liver, intestines, kidney and skin. In vitro studies have shown that cholesterol can be converted to the epoxide non-enzymatically, whereas the palmitate ester may require enzyme activation.

Substrate specificity appears to be critical in the metabolism of physiological lithocholic acid derivatives by human intestinal microflora. Lithocholic acid-3α-sulfate is completely metabolized by flora obtained from a donor on a high beef-fat diet, and the major metabolites identified appear to be mono-unsaturated derivatives of cholanic acid. When taurolithocholic or lithocholic acid is used as a substrate, a more diverse pattern of metabolites is obtained but only 25-40% of the substrate is metabolized.

Human intestinal microflora from different sources have been tested for their ability to metabolize the sulfate ester of LA. In addition, characterizations of the microflora correlate somewhat with the in vitro metabolism since higher numbers of bacteroides are present in flora which biotransforms the substrate completely, whereas higher numbers of aerobic lactobacilli are found in microflora obtained from a vegetarian donor. The latter flora metabolize

this substrate to a much lower extent and may indicate that the lack of sulfatase in the feces may result in the presence of less toxic metabolites of LA in the colon.

Several azo dyes have been incubated with individual strains of human anaerobes to study their ability to reduce the dyes and produce mutagenic metabolites. Culture filtrates of methyl orange incubated with Bacteroides fragilis, Fusobacterium sp. or human mixed flora contained mutagenic activity. One metabolite, N-N-dimethyl-p-phenylenediamine, has been tentatively identifi and is mutagenic in the Ames' assay, whereas another product, sulfanilic acid, is not.

Administration of 2-AA to rats results in enhanced DNA and protein synthesis in liver and large intestine. The similarity of macromolecular synthesis profiles observed in liver and colon as well as the difference in this profile in the jejunum suggests that 2-AA may also be carcinogenic to the colon. Metabolites of 2-AA isolated from the feces of animals fed carcinogens were mutagenic and this activity decreased with time.

Significance to Biomedical Research and the Program of the Institute: The metabolic activation of carcinogenic or cocarcinogenic precursors is an important area for understanding the formation of endogenous carcinogens in humans. Metabolic studies with enzyme systems from various portions of the digestive tract will assess organotropic effects of in situ formation of carcinogens, and bacterial mutagenesis evaluation of products will assist in the rapid identification of compounds which may pose an environmental hazard to man.

Proposed Course: Research will continue on the relationship of endogenous chemicals to carcinogenesis; these include: (1) Investigate the role of host tissue enzymes in activation and detoxification of endogenous carcinogens. Emphasis will be placed on mixed function oxidase systems of microsomes derived from liver and small and large intestines. (2) Investigate the role of bile acids and sterols in the context of endogenous carcinogenesis. Emphasis will be placed on the interaction between chemicals and microflora. (3) Investigate the role of microflora in the process of endogenous carcinogenesis. Emphasis will be on metabolism studies. (4) Investigate the role of intestinal epithelial cells in the metabolism of luminal substrates.

Project 8

Title: Bioassay Research

Contractor's Project Manager: Dr. Richard Cardy

Project Monitor (NCI): Dr. Richard A. Griesemer

Objectives: The major objective of this program is to identify the potential carcinogenicity of compounds selected for test, and to use all available resources in a program of research directed at improving the efficacy of in vivo animal assays and at establishing a clearer understanding of how the animal bioassay relates to in vitro systems now under development and, ultimately, to human cancer.

Major findings: Twenty-one compounds have been tested in both rats and mice at FCRC in the past three years and tissues are in the hands of consulting pathologists for evaluation. Seventeen studies involving 10 compounds are complete through pathological evaluation and are in various stages of reporting. Those compounds are: Azobenzene (rats and mice), 2,4,6-trichlorophenol (rats and mice), Perthane (rats and mice), Methyl parathion (rats and mice), 2-chloroethyl trimethylammonium chloride (rats), Phthalic anhydride (rats and mice), Trimethyl aniline (rats), Piperonyl butoxide (rats and mice), Piperonyl sulfoxide (rats and mice), and 4-chloro-o-toluidine (mice). Of these compounds, four (Azobenzene, 2,4,6-trichlorophenol, 2,4,5-Trimethyl aniline, and 4-chloro-o-toluidine) show evidence (statistically unverified as yet) for carcinogenesis in either rats or mice. It is anticipated that all pathology will be complete during the summer. Full development of the data through writing of technical reports will depend on the data evaluation capabilities of CBDS.

A collaborative study with Endogenous Formation of Carcinogens evaluating the effect of an all meat diet on incidence of induced colon cancer has been completed and, within the framework of the experiment, revealed no statistically significant effect on tumor incidence, size, location, or malignancy.

A study has been completed in collaboration with Basic Research in which pathological evaluation was conducted on lesions induced by intravenous administration of a BCG-based immunoadjuvant.

Pathology is complete on the rat comparative mammary tumor study in collaboration with Endocrine Carcinogenesis. There are striking differences in incidence of induced mammary carcinoma in the seven strains examined and apparently significant differences in morphological characterization of the tumors.

Significance to Biomedical Research and the Program of the Institute: A major objective of Bioassay Research at FCRC is interaction with other scientific groups at FCRC as a means for clarifying the interrelationships of many concepts in the development of a consistently predictive "test system." What makes the FCRC program of particular significance to the cancer program as a whole is its capability for conducting methodological research of great breadth. At Frederick, there exists scientific expertise to carry on, at the highest levels, all forms of carcinogenesis and basic research which are applicable to the development of the most useful bioassay systems. Located at Frederick also is a large well-designed, safe animal test facility which can be devoted in whole to the long-term animal test aspect of bioassay research. In short, Frederick has both the intellectual capacity and the hardware to conduct definitive carcinogenesis bioassay developmental research, and a program is now operative that should realize that goal.

Proposed Course: Five compounds have been agreed upon by the NCI and FCRC for Bioassay "testing." These compounds are: N,N-diallyl-2-chloroacetamide, 1,3-dichloropropene, Cyclohexanone, Styrene oxide, and Benzyl chloride. In addition, a definitive bioassay of the insecticide mirex is planned, along with a study designed to demonstrate interaction of four important environmental pollutants at low levels. Those substances are mirex, polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and chloroform.

Project 9

Title: Synthesis and Analysis of Chemicals for Carcinogenicity Studies

Contractor's Project Manager: Dr. Walter L. Zielinski, Jr.

Project Monitor (NCI): Dr. Peter P. Roller

Objectives: A major requirement of the interdisciplinary program at FCRC is the capability of preparing and characterizing a variety of carcinogens for use in the other research areas as well as a need to provide analytical expertise and support to the other program areas. Important to the overall research needs is the synthesis of carcinogens, known or potential metabolites of carcinogens, postulated or known products of carcinogen-cellular interactions and other organic chemicals of high purity.

The chemistry staff has had an active collaboration with investigators in the other areas of research at FCRC. This collaboration is essential and is expected to continue.

Major Findings: A major advance was made in the preparation of derivatives of carcinogenic polycyclic aromatic hydrocarbons with the development of a new synthetic route to benz[a]anthracenes in three steps, and to derivatives of 7,12-dimethylbenz[a]anthracene in four steps. This represents a substantial decrease in the number of steps required by conventional routes of synthesis. The development of high temperature nematic liquid crystals for novel GLC separations of isomeric polycyclic aromatic hydrocarbons was extended to GLC-mass spectrometry and to separations of metabolites of benzo[a]pyrene and bile acids. Other analytical advances have included the development of: (1) spectroscopic (UV, AAS, MS, IR) techniques combined with quantitative in situ TLC elution for analysis and characterization of trace carcinogens, (2) high pressure liquid chromatography methods for accelerated assay of methylated purines and N^1-methylnicotinamide in urine, (3) a chemical ionization mass spectrometry data scheme for the identification of bile acid metabolites, (4) a simple disposable containment system for the safe handling of chemical carcinogens, and (5) a variety of analytical methods for collaborative studies with scientists in other sections of the Chemical Carcinogenesis Program and the Chemotherapy Fermentation Laboratory.

Other accomplishments have included: (1) the completion of in vivo and in vitro studies on dimethylnitrosamine (DMN) using a deuterium isotope effect protocol, (2) the development of an in vitro assay for DMN methylation using RNA as an acceptor molecule, (3) the analysis of methylated purines in rat urine following treatment with radio-labelled carcinogenic methylating agents, and (4) the initiation of a collaborative evaluation of the potential hazard of various hair dye components and their oxidation products.

Significance to Biomedical Research and the Program of the Institute: Adequate bioassay research can only be done with proper analytical chemistry backup for the identification of chemicals being administered, analysis of their purity, and indication of the length of time they are stable under conditions of storage and administration. This project provides that type of support.

An expanding activity of this project is the synthesis of chemicals needed to study the metabolism of carcinogens. It is anticipated that in the future the project will develop the capability for synthesizing radioisotopically-labelled chemicals for laboratory investigation.

Proposed Course: Work in the areas of synthesis, analysis and method development will continue. This work will include:

1. Synthesis of organic chemicals of high purity will be required to support studies of metabolism of carcinogens. In some cases, isotopic labelling will be necessary.

2. Analytical determinations to support bioassay and other research include: (a) physical characterization of materials by such means as UV, IR, melting point, boiling point, refractive index, and qualitative identification and quantitative determination of the nominal ingredient or ingredients of each specific substance; (b) detection and partial characterization of impurities present in each substance; (c) determination of the rate of loss of each substance when mixed in animal feeds, periodically checking on the efficiency of the chemical/feed mixing processes employed in the bioassay contracts by analyzing aliquots; (d) periodically analyzing samples of animal feed and bedding for pesticides and the principal mycotoxin residues.

3. (a) Develop new methods using GLC, HPLC, TLC, column chromatography and electrophoresis for isolation, detection and measurement of chemicals for studies at FCRC and for collaboration with NCI and other carcinogenesis research facilities. Develop liquid crystals (synthesis) and test their separation properties on GLC for the resolution and quantitation of carcinogens, their metabolites, and chemicals of significance to the carcinogenesis program. (b) Detect and partially characterize impurities in each chemical substance, including their number and approximate percentages by the generation of chromatographic retention data (LC, TLC, GLC) in two or more chromatographic systems.

Project 10

Title: In Vitro Carcinogenesis

Contractor's Project Manager: Dr. Roman J. Pienta

Project Monitor: Dr. Virginia C. Dunkel

Objectives: The primary objective of this project is to develop standardized in vitro bioassay methods for detecting potential carcinogens which present a high degree of hazard for man and the use of these systems for studying the mechanism of action of chemical carcinogens. Emphasis is placed on defining standard experimental conditions and endpoints so that the system is reproducible, rapid, and valid and can be readily used in other laboratories.

Major Findings: Standard In Vitro Carcinogenesis Bioassay: A hamster embryo cell carcinogenesis bioassay was standardized by using pretested aliquot samples of primary cultures cryopreserved in liquid nitrogen as the source

of target and x-irradiated feeder cells. To verify the reliability of the system, it was tested with a number of standard reference chemicals having known carcinogenic activities. A very high correlation (91%) of morphological transformation with their reported carcinogenic activity was obtained when 88 coded reference chemicals were tested. Transformation was observed with most carcinogens, and no false positives were observed when cells were treated with noncarcinogens, nor was spontaneous transformation ever observed in control cultures treated with solvent or culture medium alone. Several carcinogens requiring metabolic activation did not induce morphological transformation. One of these, AAF, was metabolized to an active form capable of transforming target cells when tested in the presence of hamster liver microsome enzymes. This modification is being exploited in efforts to minimize or eliminate false negative responses. The bioassay was used in collaborative studies designed to identify synthetic organotropic carcinogens and to assess the carcinogenic potential of several aromatic diamines, food additives and endogenous materials produced in the gut. Preliminary solubility and toxicity tests were performed on twenty coded compounds currently under investigation for their carcinogenicity in animals, in our efforts to evaluate the predictive value of the in vitro system when it is used for prescreening chemicals with unknown carcinogenic potential.

The INVIS Computer Program for storing, retrieving and handling results from the hamster cell bioassay is completely on line and will be used as the prototype for computerizing a data base in the coordinated In Vitro Carcinogenesis Program of the National Cancer Institute.

Criteria for In Vitro Malignant Transformation by Carcinogenic Chemicals: When rigorous criteria are employed, altered morphology of colonies in culture can be used as an early index of transformation. Criteria other than morphology were compared with tumorigenicity to determine their relevance to malignant transformation. Results with hamster cells show that the ability of transformed cells to grow in semisolid agar appeared to be the most relevant criterion. In all cases, transformed cells that grew in agar induced sarcomas when injected into suckling hamsters. Other criteria showing good but not absolute correlation include the increase in cloning efficiency, extended life span, loss of contact inhibition and growth in factor-free medium or medium supplemented with a serum substitute. None of the parameters alone can be used as an absolute criterion for malignant transformation. Moreover, they cannot readily be used as early markers for transformation.

Effects of Tumor Promoting Agents on Transformation: The latent period to morphological transformation was the same when mass cultures of hamster embryo cells were treated with 3-methylcholanthrene (3MC) alone or simultaneously with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Cells treated with 3MC alone grew in semisolid agar and induced tumors in hamsters. Cells treated with 3MC and TPA formed tumors in hamsters but grew in agar only after 20 additional passages. None treated with TPA alone grew in agar or induced tumors but they did grow 10 generations longer than control cells treated with DMSO. The effects of TPA on cloning efficiency, thymidine-^{3}H incorporation and binding of 3MC-^{3}H were investigated.

Binding of Aromatic Hydrocarbons to human Embryo and Hamster Embryo Cells in Culture: It was observed that the binding of tritiated dibenz(a,h)anthracene, dibenz(a,c)anthracene and 7,12-dimethylbenzanthracene to cellular DNA, RNA and protein of human embryo cells increased with the number of cell generations until generation 70. Moreover, increasing serum concentration from 5% to 20% resulted in a 4-fold decrease in dibenz(a,h)anthracene-^{3}H binding to human embryo cell protein. A 4-fold increase in the binding of this carcinogen to cellular protein was found when cells were treated at confluence rather than at 50% confluence.

Metabolic Activation: N-2-acetylaminofluorene (AAF) can be metabolized in the presence of liver microsomes and NADPH to form metabolites capable of transforming hamster cells. Major products of hydroxylation or deacetylation are dependent upon the species from which liver extracts are prepared. A procedure employing high pressure liquid chromatography for effectively separating N-hydroxy-AAF from ring hydroxylation products to facilitate the determination of the N-hydroxylation reaction by enzymes from human and hamster embryo cells was developed. AAF deacetylase activities from hamster liver microsomal preparations were 2- to 6-fold greater than those reported in other species. Preliminary experiments show that diethylnitrosamine can be activated by liver microsomes.

Studies with Human Cells: In earlier studies, 47 cultures were isolated from semisolid agar after treatment of human cells with either 3MC or MNNG. Cryopreserved samples are being tested for their ability to grow in agar and other characteristics of transformed cells. More recently, early passage human cells were synchronized and sequentially treated with MNNG and 4NQO. Colonies in agar were observed after three cycles of treatment. Subcultured samples of treated and untreated cells were cryopreserved and are being evaluated for malignancy in athymic nude mice and immunosuppressed hamsters and other in vitro criteria for transformation.

Significance to Biomedical Research and the Program of the Institute: A major objective of the National Cancer Program is the identification of potentially carcinogenic chemicals posing a hazard to man so that these agents can be removed from the environment. This becomes more important as increasing numbers of chemicals are introduced each year, thereby increasing the number of potentially carcinogenic chemicals which should be identified. The in vitro carcinogenesis project at FCRC emphasizes the development of reliable short-term carcinogenesis bioassays that could be used for prescreening chemicals and selecting those requiring further testing in animals. The hamster embryo cell in vitro carcinogenesis bioassay shows promise of being such a system because of the high degree of response concurring with conventional in vivo bioassay. The system can also be used to study the mechanism of action of carcinogens so that steps might be devised to inhibit or interrupt the process and thus reduce the incidence or modify the course of cancer.

Proposed Course: Efforts will be continued to identify optimal experimental conditions for obtaining maximum susceptibility to transformation of both human cells and cells of experimental animals by carcinogens. Transformation by a broader range of classes of chemical carcinogens will be investigated

to establish the usefulness and limitation of the system. Continued emphasis will be placed on enhancing the sensitivity of the system to eliminate the false negative responses seen with several carcinogens by incorporating metabolic activation, or tumor promoters, or by modifying the experimental design and cultural conditions of the system. This also would allow the system to detect weak carcinogens and help to assign potencies of chemicals with unknown carcinogenic potential. The metabolism of selected carcinogens by microsome enzymes from several mammalian species, including man, will be continued to gain insight into the process of carcinogenesis and to make the bioassay more relevant to identifying carcinogens hazardous to man.

Project 15

Title: Procedures for Safe Handling of Chemical Carcinogens

Contractor's Project Manager: Dr. Eric B. Sansone

Project Monitor (NCI): Dr. Larry Keefer

Objectives: The objectives of this project are to assist in formulating effective measures to prevent cancers of occupational origin in laboratory workers; to provide adequate protection to the general environment during the course of research programs involving the acquisition, storage, manipulation, and disposal of known or suspect chemical carcinogens; and to ensure that the integrity of experimental programs is not compromised by chemical contamination.

Major Findings: Guidelines for safe practice have been drafted for auramine, benzidine, carbon tetrachloride, chloroform, DDD, DDT, cycasin, dibenz[a,h]-acridine, dibenz[a,j]acridine, fluorescein and mirex. A draft code of safe practice, prepared with Dr. P. F. Swann for the IARC, has received initial review by the IARC.

Feed blending, feeding, and other maintenance and handling procedures associated with the bioassay program have been modeled. The feeding of agar-base gelled diet to animals housed in solid and wire-bottom cages was monitored in collaboration with the Massachusetts Institute of Technology. Modeling also included the subdivision of solid and liquid carcinogens, venting of separatory funnels, injection of materials through septa, and recovery of samples by scraping from thin-layer chromatographic plates.

Common laboratory glove materials were tested with carbon tetrachloride and chloroform to validate the permeability cells and procedures to be used in testing the efficacy of such gloves for carcogenesis research. The cells and experimental procedures were satifactory. However, all glove materials tested allowed breakthrough within 7 minutes of exposure to the test chemical.

A gas flow test apparatus has been assembled to study the absorption of contaminant vapors by activated charcoal. Initial experiments are being performed to calibrate the apparatus.

Significance to Biomedical Research and the Program of the Institute: Numerous laboratories throughout the world are working with chemical carcinogens. Until now, there has not been a central laboratory for the purpose of developing and analyzing safety techniques for the protection of carcinogenesis research workers and the surrounding community from hazardous exposure to chemical carcinogens. This project is designed to meet this need.

Proposed Course: Areas of current and future concern to the chemical carcinogenesis safety program include: (1) Developing guidelines for safe practice: a) characterize the state of the art for handling of chemical carcinogens by critically reviewing the relevant published and unpublished information and preparing guidelines for safe practice; b) when deficiencies are identified, propose alternative approaches to overcome them, and select and investigate the most promising approaches; c) continually reevaluate and reassess guidelines to update recommendations and revise priorities; d) recommend means for eliciting adherence to safe practices and assess their effectiveness; e) upon NCI request, make visits to specific facilities to assess carcinogen safety programs and atttitudes. (2) Investigating contamination potential of laboratory procedures: a) investigate selected, common laboratory procedures and characterize the means and extent of chemical dissemination during various laboratory operations; b) assess the risk to laboratory personnel, to the general environment, and to nearby experiments resulting from the use of chemicals. (3) Investigating effectiveness of personal protective equipment. (4) Investigating effectiveness of activated charcoal for the removal of gaseous contaminants. (5) Investigating degradation, decontamination, and disposal of chemical carcinogens.

Project 16

Title: Endocrine Carcinogenesis

Contractor's Project Manager: Dr. Douglas H. Janss

Project Monitor (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objective of this research has been to investigate dimethylbenz(a)anthracene carcinogenesis and the mechanism of action of the steroids in a rat mammary gland model and to utilize human breast tissue in vitro to compare animal and human data.

Major Findings: The rat DMBA mammary tumor system in which tumors are rapidly induced under specific conditions serves as the foundation for the experimental approach. It has been determined that the age, strain, and hormonal status of the rat must be such that the mammary epithelial cells are in a state of high DNA synthesis and low RNA and protein synthesis for DMBA to initiate tumor formation. In this state, DMBA is metabolized to chemically reactive derivatives and rapidly bound in significant amounts to DNA. Experiments indicate that a mammary stem cell capable of either responding to estrogen stimulation by cell division or responding to progesterone and prolactin stimulation by ultimate differentiation may be the target for the DMBA carcinogenic action. The binding of DMBA to the DNA of this mammary epithelial cell apparently results in the alteration of genetic

information and an initial depression of both DNA and RNA synthesis. This depression is followed by a period of DNA and RNA synthesis which is greater than that found in untreated cells. DMBA treatment leads to the retention and synthesis of significant quantities of a PolyA-RNA, while mammary epithelial cells of untreated animals show both a loss and a decreased synthesis of this class of RNA. The expression of this DMBA-altered genetic information is manifest by either the continued synthesis of a specific mRNA or the synthesis of a new mRNA. This mRNA could conceivably prevent the ultimate differentiation of the cell and in turn lead to continued cell division, the ultimate result of which would be a mammary adenocarcinoma.

A rat mammary epithelial cell culture system which responds to normal physiological serum levels of insulin, aldosterone, estradiol-17β, progesterone, and prolactin has been established. These hormones are capable of stimulating DNA, RNA, and specific protein synthesis in such a manner that this culture system mimics the normal growth of mammary epithelial cells in vivo. Conditions necessary for the metabolism and binding of DMBA to DNA in this culture system have been found to be similar to those necessary for the occurrence of these events in vivo. Furthermore, in vitro responses to DMBA exposure result in alterations in DNA and RNA synthesis which are comparable to those found in the mammary gland after the in vivo administration of DMBA. Cultures of mammary fibroblasts do not respond to either hormone or DMBA addition to the culture medium. A system for the growth and maintenance of normal human breast epithelial cells in culture "free" from contaminating friboblasts has been developed and the hormonal requirements for optimal cell growth in culture are being examined.

Significance to Biomedical Research and the Program of the Institute: This project is undertaking a number of important problems including the definition of the conditions under which mammary tissue is susceptible to malignant transformation, the role of hormones in this process, and the definition of the mechanisms whereby steroids may be carcinogenic either through normal steroid action or through established carcinogenic processes.

Proposed Course: Research will be initiated to investigate factors responsible for the induction of cancer in endocrine target organs of humans and experimental animals. Special emphasis shall be placed on the study of carcinogenesis in the breast. Research will include the following: (1) Development of experimental animal models for the induction of tumors comparable to those occurring most commonly in humans. (2) Determination of the susceptibility of various species and strains to carcinogenesis at different ages and under varied experimental conditions. (3) Investigation of the endocrine, metabolic, and morphologic states associated with high and low susceptibility to carcinogenesis. (4) Investigation of the conditions and mechanisms under which hormones induce cancer in experimental animals in the absence of exposure to other known carcinogens and the relevance of these observations to the development of tumors of endocrine target organs in the human. (5) Investigation of the effect of endocrine manipulation on susceptibility to carcinogenesis. (6) Investigation of the process of cell differentiation, maturation and aging in endocrine target organs. (7) Development of cell and organ culture carcinogenesis models using experimental animal and human tissues.

Project 20A

Title: Molecular Aspects of Chemical Carcinogenesis

Contractor's Project Manager: Dr. Anthony Dipple

Project Monitor (NCI): Dr. Elizabeth K. Weisburger

Objectives: The specific objective of this project is to define aspects of the process of tumor initiation which may be common to a wide range of chemical carcinogens. Emphasis is placed on defining chemical reactivity associated with carcinogenesis and identifying a critical receptor site within the cell.

Major Findings: Comparisons of the excision of DNA damage introduced by 7-bromethylbenz(a)anthracene and, the more carcinogenic homologue, 7-bromomethyl-12-methylbenz(a)anthracene did not reveal any major differences. Thus, carcinogenic potency probably does not residue in an ability to introduce intrinsically irreparable damage into cellular DNA.

Excision of 7-bromomethylbenz(a)anthracene-DNA adducts and cell survival has been examined for normal human cells and for repair-deficient xeroderma pigmentosum cells. In general, these properties parallel those reported in the literature for the responses to UV damage, except for the line XP2. N^6-(Benz[a]anthracenyl-7-methyl)adenine residues are excised more readily than N^2(benz[a]anthracenyl-7-methyl)guanine residues in most of the human cells.

The study on the rates, dose-dependence, extents, and specificity of excision in rodent cells has shown that excision can be inhibited at high carcinogen concentrations, and that even at low concentrations DNA replication occurs on an extensively damaged template. It is conceivable that the rate at which DNA is damaged is more relevant to tumor initiation that the final extent of the reaction. Tumor-initiating activity may also depend on the selectivity exhibited by the carcinogen for reaction at different locations within the genome. It has been shown by two different chromatin fractionation procedures that aralkylating agents of different carcinogenic potencies exhibit different selectivities in their reactions with DNA. The more carcinogenic agent distributes itself in a more random fashion. Experiments have been designed to examine the influence of reactivity on product distribution for reactions of aralkylating agents with nucleic acid constituents. Some progress has been made, although this phenomenon is far from being understood.

Studies of the mechanism of metabolic activation of 7,12-dimethylbenz(a)-anthracene (DMBA) have yielded some valuable information. The binding of DMBA to DNA is being examined in mammalian systems and in in vitro activating systems in current use in rapid screening systems. The products obtained from DMBA-DNA from mouse embryo cells are chromatographically separable from those obtained from DNA treated in vitro with either the K-region oxide, 7-bromomethyl-12-methylbenz(a)anthracene, or 7,12-dibromomethylbenz(a)-anthracene, and from those obtained when 7-hydroxymethyl-12-methyl(a)-anthracene is bound to DNA in cultured cells. DMBA-DNA products are

photosensitive and after comparison with the photosensitivities of various model compounds it can be concluded that the DMBA-DNA products should contain either an intact benz(a)anthracene aromatic system or a dimethyl-anthracene system. The fluorescence exitation and emission spectra of the DMBA-DNA products further define the mechanism of DMBA activation and show that this occurs through DMBA 3,4-oxide and not through a diol-oxide.

Significance to Biomedical Research and the Program of the Institute: One of the goals of the National Cancer Institute is to prevent cancer in man through the identification and detection of environmental carcinogens. Basic research aimed at identifying common features in the mechanism of action of chemical carcinogens should provide a rational basis for improvement in existing procedures for identification and detection of environmental carcinogens.

Proposed Course: Research will be conducted in an attempt to define aspects of the process of tumor initiation which may be common to a wide range of chemical carcinogens. Emphasis will be placed on the interaction between a carcinogen or a metabolite and critical receptors within the cell. Research will include: (1) Studies of carcinogenic activities of aralkylating carcinogens by comparative studies with chemicals of known properties. (2) Studies of chemistry and reactivity of carcinogens. (3) Studies of hydrocarbon-DNA binding through investigations of photo-sensitivities of products and through comparisons of binding products in different susceptible tissues. (4) Investigation of specificity of chemical reactions with DNA in various subcellular chromatin or DNA fractions. (5) Investigation of differences in excision repair responses which may be related to the expression of their carcinogenic activities.

Project 20B

Title: Cofactors in Chemical Carcinogenesis

Contractor's Project Manager: Dr. Winifred Palmer

Project Monitor (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives are to determine the mechanism by which tumors elicit elevations in serum Hp and to establish the use of serum Hp levels as a monitor for the interactions between phagocytic cells and tumor cells.

Major Findings: The ability of macrophages to metabolize 7,12-dimethylbenz-(a)anthracene (DMBA) has been studied and compared to the metabolism of DMBA by mouse embryo cells. Isolated macrophages were incubated in culture dishes in the presence of ^{14}C-DMBA. During the first two hours of incubation, over 90% of the radiolabel disappears from the medium. Approximately one-third of the DMBA is taken up by the cells and the remainder adheres to the surface of the plastic dishes. During the following 20 hours, low levels of radioactive material are slowly released from the cells into the medium. At 24 hours, more than 90% of the radiolabel within the cells is associated with DMBA. The DMBA present in the medium decreases steadily with time and is present in negligible quantities at 24 hours. About 60 to 70% of the

radioactivity is associated with two metabolites in the medium with over 40% of the total counts associated with the less polar peak (Rf = 0.35).

The radioactivity profile of the medium from mouse embryo cells differed considerably from that of macrophages. The more polar metabolite comprised over 50% of the total counts. The peak which predominated in the macrophage culture medium was not present. The radioactivity profiles of cellular extracts were comparable to those of macrophages.

The major nonpolar DMBA metabolite produced by macrophages is chemically distinct from available DMBA derivatives (7-hydroxymethyl-7,12-DMBA and 8,9-dihydrodiol-7,12-DMBA).

Significance to Biomedical Research and the Program of the Institute: Numerous studies have shown that macrophages can exert cytotoxic effects on tumor cells in vitro. However, the consequences of interactions between phagocytic cells and tumors within the host are poorly understood. The number of leukocytes associated with tumors can be measured by manipulation of excised tumors. However, excision provides a single measure during the entire course of tumor development. The elucidation of the effects of these interactions over time is essential to the understanding of the host defenses against tumors and to the application of immunotherapeutic measures which rely on the stimulation of the reticuloendothelial system. Using serum Hp as a marker for phagocytic activity, the relationship between macrophage activity and tumor progression and regression can be studied. Serum Hp concentrations can be readily determined from small amounts of serum without altering the physiological condition of the animal or affecting tumor development.

Proposed Course: Research will be carried out to investigate the role cofactors may play in the early stages of the carcinogenic process. Research will include: (1) Investigate the role of macrophages in this process. (2) Investigate the role of promoters on macrophage metabolism. (3) Investigate the role of products of macrophage metabolism.

Project 20D

Title: Mechanisms of Nitrosamine Carcinogenesis

Contractor's Project Manager: Dr. William Lijinsky

Project Monitor (NCI): Dr. Elizabeth K. Weisburger

Objectives: The main objective of the program is to explore the relationship between cancer in man and nitrosamine exposure. This involves studies of the formation of N-nitroso compounds, including mechanisms and kinetics. Studies of the distribution of precursors of N-nitroso compounds included development of new analytical procedures for secondary and tertiary amines, nitrites, and N-nitroso compounds. Those for secondary amines are quite well established and are being applied to many environmental materials; those for tertiary amines are proving difficult and have only had limited application. The problems posed by the finding of transnitrosation as a common

phenomenon are being explored, particularly in connection with N-nitroso compounds in foods. Such N-nitroso compounds, even though themselves noncarcinogenic, might transfer their nitroso group to other amines. Also of interest is the relevance of transnitrosations which might occur in man's stomach.

Major Findings: The significance of formation of N-nitroso compounds in vivo has been investigated by feeding combinations of secondary and tertiary amines of environmental interest, especially drugs and agricultural chemicals together with nitrite to rats in drinking water. In a few instances, the combination of the amine and nitrite led to a significant incidence of induced tumors, indicating a large measure of formation of N-nitroso compound in the stomach.

About 80 compounds, which can be classified in several groups, have been tested in rats at equimolar doses, and large variations in carcinogenic activity have been observed. Various substituents have enhanced or reduced carcinogenic activity of a parent molecule, depending on the position of substitution. Deuterium or methyl substitution in the alpha positions reduce carcinogenic activity. These results indicate that cleavage of an alpha carbon-hydrogen bond is a rate-limiting step in carcinogenesis by nitrosamines. Among cyclic nitrosamines, conformation of the molecule is an important factor in determining carcinogenic activity.

Mechanistic studies of the formation of N-nitroso compounds include the nitrosation of tertiary amines which seems to be second order in nitrate. Transnitrosation of aliphatic nitrosamines has important consequences for human exposure to N-nitroso compounds through formation of carcinogen N-nitroso compounds from noncarcinogenic ones that might be present in food or other ingested materials.

Analytical methods have been developed for separating and estimating secondary and tertiary amines that might be nitrosamine precursors in foods, tobacco smoke and other materials.

To investigate the mechanism of carcinogenic action of N-nitroso compounds, several of them have been synthesized and labelled with carbon-14 or tritium. These compounds show distinct organ specificities between one and another. It has been shown that high doses in rats and hamsters are metabolized less efficiently than the smaller doses which are more effectively carcinogenic on repeated application.

Significance to Biomedical Research and the Program of the Institute: Nitrosamines represent the most diversely acting group of chemical carcinogens, including models of most human cancers. The probability that they can form in the environment or in vivo from common precursors makes it likely that they are involved in human cancer. Prevention or reduction of their formation could reduce human exposure to carcinogens. N-nitroso compounds are readily available; their chemistry is largely unexplored, and studies of their biochemistry which can be related to induction of cancer by them might reveal information having a bearing on the mechanism of carcinogenesis in general.

Proposed Course: Future research will include: (1) Investigation of the formation of N-nitroso compounds. (2) Investigation of structure activity relationship between N-nitroso compounds and the process of carcinogenesis. (3) Investigation of differences in potency and organ and species specificity of highly similar nitroso compounds. (4) Investigation of the distribution of nitroso compounds and their precursors in the environment.

Date Contract Initiated: June 26, 1972

Current Annual Level: $6,866,357

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER) (NO1-CP-33278)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. David B. Clayson

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The bioassay of suspected chemical carcinogens, definition of the biological and chemical mechanisms of the carcinogenic process and the analysis of trace levels of environmental carcinogens are three principal research aims under this contract.

Major Findings: A. Hydrazine Carcinogenesis--During the past three years, approximately 10 new hydrazine analogues were shown to be carcinogenic in laboratory animals in this Institute. Among these, the most notable are: β-Phenylethylhydrazine sulfate (Nardil), presently used to treat mental depression, succinic acid, 2,2-dimethylhydrazide, a widely used herbicide, N-acetyl-4-(hydroxymethyl)phenylhydrazine, an acetylated derivative of 4-hydroxymethylphenylhydrazine, an ingredient of the commonly eaten, cultivated mushroom Agaricus bisporus, and N-methyl-N-formylhydrazine, a constituent of the edible, false morel Gyromitra esculenta.

Since a large segment of the human population in the United States is exposed to these chemicals, it is apparent that from an environmental viewpoint, they constitute a formidable hazard.

Three hydrazine derivatives which induce large bowel cancers were also tested. Two of these compounds, which originate in nature, are 1,1-dimethylhydrazine, a tobacco ingredient, and methylhydrazine formed from a chemical in the edible wild mushroom Gyromitra esculenta. In addition, both compounds are manufactured and used in rocket fuel. The other chemical, trimethylhydrazine HCl, is not used in the environment. All three induced significant incidences of tumors in the large intestine of laboratory animals.

B. Other Bioassays--Highlights of the bioassay program incluce the following: (a) Nitrosamines are usually considered to be systemic carcinogens and to act in tissues remote from the application site. It has been demonstrated, however, that nitrosamines with specific molecular structures are primarily

locally-acting carcinogens. For example, N-nitroso(1-acetoxypropyl)-N-propyl-nitrosamine (1-APPN) induced a high incidence of malignant Schwannomas, fibrosarcomas and mammary carcinomas after subcutaneous administration for life. N-Nitrosobis(2-hydroxypropyl)amine (BHP) and N-nitrosobis(2-acetoxy-propyl)amine (BAP) induced a large number of trichoepitheliomas of the lip and vaginal papillomas following weekly topical application of these two compounds. All three nitrosamines, 1-APPN, BHP, and BAP exerted, in addition, a systemic neoplastic effect.

(b) Injected nitrosamines, e.g., methylamylnitrosamine, caused an acute inhibition of [^{3}H]thymidine uptake by epithelial DNA of the rat esophagus. For six nitrosamines, this inhibition was correlated with carcinogenicity for the esophagus. The effect was also observed when nitrosamines were fed for 4 weeks and when the isolated whole epithelium was incubated with nitrosamines. The latter finding suggests that the enzymes responsible for metabolic activation of nitrosamines are present in the esophagus. This approach offers promise as an in vivo bioassay for detecting factors responsible for human esophageal cancer.

(c) N-nitrosobis(2-oxopropyl)amine (BOP) is a potent pancreatic carcinogen in the hamster. BOP induced, in a relatively short period of time, a high incidence of pancreatic ductal cell tumors in doses considerably lower than those needed with the previously demonstrated pancreatic carcinogen N-nitroso-bis(2-hydroxypropyl)amine (BHP) and N-nitrosobis(2-acetoxypropyl)amine (BAP). The pancreatotrophic effect of these three compounds was found to be directly related to the in vivo formation of N-nitroso(2-hydroxypropyl)(2-oxopropyl)-amine (HPOP), which was formed more readily from BOP than from BHP and BAP. The assumption that HPOP may be a proximate pancreatic carcinogen was supported by the observation that N-nitroso-2,6-dimethylmorpholine, which has also been reported to be a pancreatic carcinogen, produced HPOP as an in vivo metabolite. The chemical structure of this metabolite is of specific interest, since it can occur in a cyclic form resembling the pyranose form of glucose.

(d) Studies in the area of food additives and contaminants concerned hexa-chlorobenze (HCB), which induced a high incidence of hepatomas and vascular tumors in hamsters fed HCB for life. Other studies with DDT resulted in induction of few tumors in hamsters. The incidence was similar in controls and DDT-treated animals. Another series of studies on the acute toxicity of various chlorinated pesticides in hamsters has involved tests of 10 pesticides thus far. The first results of these acute toxicity experiments in hamsters showed that this species is as sensitive as the rat to this family of compounds.

Mechanisms of Carcinogenesis--(a) The naturally occurring hydrazine compounds, agaritine and gyromitrin, are found in the mushroom families of Agaricus and Gyromitria, respectively. While these compounds can be extracted from the mushrooms, the needs of the bioassay program are better met by synthesizing them. The major difficulties involved in synthesizing agaritine have been overcome. The yields are much improved over those reported by previous workers (less than 1%).

(b) The carcinogen methylhydrazine is formed from gyromitrin in the mouse stomach. A gas-liquid chromatographic method for estimation of methylhydrazine and other hydrazines in aqueous environments has been developed. This method is equally valuable for stomach contents, urine, blood, etc.

(c) A current investigation demonstrated for the first time that pyridoxine hydrochloride (vitamin B6) reversed the toxic action of four substituted hydrazines: ethylhydrazine HCl, n-butylhydrazine HCl, methylhydrazine and β-N-[γ-L(+)-glutamyl]-4-hydroxymethylphenylhydrazine. Studies on the possible inhibition of tumor induction by vitamin B6 are presently underway.

Significance to Biomedical Research and the Program of the Institute: The Eppley Institute has provided over the years a considerable output of major research results in chemical carcinogenesis and represents a prime national resource in which chemical, pathological and biological aspects of carcinogenesis are studied in an integrated progam.

Proposed Course: This contract will continue to support the study, improvement, and conduct of bioassay techniques and research into chemical mechanisms of the carcinogenesis process, as well as analytical studies of environmental carcinogenesis. Work will continue and expand as outlined above.

Date Contract Initiated: March 19, 1968

Current Annual Level: $3,100,000

NEW HAMPSHIRE, UNIVERSITY OF (NO1-CP-55675)

Title: Preparation of Various N-Nitroso Compounds

Contractor's Project Director: Dr. Paul R. Jones

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) Develop improved synthetic methods for carbon-labelled heterocyclic nitrosamines of potential interest in carcinogenesis research. Such compounds might include N-nitrosopiperidine, N-nitrosopyrrolidine and others at the direction of the Project Officer. (2) To prepare pure samples of a number of unlabelled N-nitroso compounds, to be identified by agreement with the Project Officer, and deliver them safely to the Carcinogenesis Standard Reference Compound Bank. (3) To investigate other synthetic approaches to radiolabelled N-nitroso compounds as may be mutually agreeable to the Principal Investigator and the Project Officer.

Major Findings: The preparation of nitrosamines from secondary amines can be accomplished readily, using nitrosyl chloride and a base. This method of synthesis is particularly convenient for isotopically-labelled amines since the nitrosamine is formed in high yield and purity. The second convenient method of synthesis from this work involves the conversion of cyclic nitrosamines, via their anion, to new substituted nitrosamines. The reaction is

highly stereoselective which suggests the potential of asymmetric syntheses by this method. This procedure would be of greatest importance in the syntheses of acids such as proline and pipecolinic acid or bicyclic nitrosamines such as tropane or pseudopelletierine derivatives. The preparation of nitrosamines by cleavage of tertiary amines with nitrosyl chloride or nitrosation of secondary amines by 1-nitrosopyridinium chloride proved unsuccessful.

The spectral properties of the nitrosamines show the strong polarization of the nitrosamine function and suggest an importance of this polarization in the metabolism and decomposition of nitrosamines.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: The investigations made under this contract contribute directly and indirectly to adding N-nitrosamines needed for biological researches to the Compound Bank of the Division. These compounds are essential for an understanding of the metabolism and mode of carcinogenesis of nitrosamines. The synthetic studies may provide clues about environmental syntheses and interconversion of nitrosamines, and structural information may suggest the nature of interactions of nitrosamines with biological systems.

PROPOSED COURSE: The synthetic studies will be continued to prepare nitrosamines, labelled and unlabelled, as needed for research in carcinogenesis. The spectral studies will be continued to obtain information about the interaction of the nitrosamine function with other functions.

DATE CONTRACT INITIATED: January 31, 1975

ANNUAL CURRENT LEVEL: $45,920

SALK INSTITUTE (NO1-CP-33405)

Title: Carcinogenesis and Control of the Cell Cycle

Contractor's Project Director: Dr. Robert W. Holley

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: The growth of normal and tumor cells is controlled in part by the genome of the cell and in part by extracellular agents. In addition to the supply of common nutrients (e.g., amino acids, sugars, etc.), the extracellular fluid contains hormones and analogous control substances, some of which influence the availability of the nutrients to the cell. Carcinogenesis changes are the relationship between the cell and the extracellular agents such that growth rates greater than normal occur. However, it has been found that some regulations of growth still remain in chemically-induced tumor cells, and the residual controls in these malignant cells is the subject of the present study. The overall objective of this study is to determine the kinds of changes that accompany chemical transformation of cells. The present project contributes to that goal, but it focuses on mechanisms whereby the growth rate in chemically-induced tumors might be checked.

Major Findings: Work has concentrated on understanding the changes in growth control that take place when 3T3 cells are transformed by benzo(a)pyrene. Transformation of these cells is accompanied by a decreased utilization of serum growth factors. This decreased utilization of growth factors accounts for the increased growth of the chemically-transformed cells in cell cultures and probably contributes to the tumorigenicity of these cells in vivo.

During the last 15 months, the interaction of serum growth factors with the transformed cells has been studied in order to understand the causes of the decreased utilization of growth factors. It has been found that one action of the serum growth factors is to bind to the plasma membranes of the cells and stimulate a sodium ion pump that is coupled to transport of nutrients into the cells. This suggests that the factors act, to some extent at least, at the cell surface.

Using radioiodine labelled epidermal growth factor to study binding of the factor to the surface of the cells, it has been found that transformation probably does not change the number of receptor sites per cell. Instead, the decreased utilization of serum growth factors is partly accounted for by a decreased affinity of the receptor sites for the growth factor. At physiological concentrations of the growth factor, the decreased affinity results in fewer molecules of factor being bound per cell. These fewer molecules have been found to have greater biological activity in stimulating DNA synthesis in the transformed cells, which also leads to a decreased requirement. It remains to be determined whether the growth factor that is bound by the transformed cells is internalized and degraded at the same rate that occurs in normal cells.

Significance to Biomedical Research and the Program of the Institute: This contract will attempt to provide more detailed information regarding the relationship between extracellular factors, membrane controls, and intracellular factors in normal and chemically-transformed cells. This can provide insight into the rates of growth of different types of cancers which can serve as a basis of considering new means of controlling malignant growth.

Proposed Course: Growth control mechanisms will be studied in various chemically-induced tumor cells and in cells transformed chemically in vitro. From past experience, it is anticipated that some of these controls will involve previously unrecognized components of serum and attempts will be made to identify these components. Attempts to identify other growth regulatory mechanisms in chemically-transformed cells will involve manipulation of the composition of the medium as well as manipulation of serum fractions. There will be a search for interactions between growth regulatory mechanisms. An attempt will be made to understand the fundamental changes that lead to changes in growth control in chemically-transformed cells.

Date Contract Initiated: June 26, 1972

Current Annual Level: $161,834

SOUTHERN RESEARCH INSTITUTE (NO1-CP-65740/BOA)

Title: Preparation of Carcinogens

Contractor's Project Director: Dr. Y. Fulmer Shealy

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To synthesize, purify, and thoroughly characterize designated chemicals required for research on chemical carcinogenesis. This project is designed to serve a dual purpose: (1) to provide the Chemical Repository of the Carcinogenesis Program with chemicals that are unavailable from other sources; (2) to provide well-characterized reference samples to investigators, through the Repository, to eliminate variability in experimental results that might be caused by impurities in inadequately analyzed specimens from various sources.

Major Findings: Two interrelated tasks, required prior to the initiation of synthesis work, were completed. Task 1 consisted of a report of the capability of laboratory facilities and safety procedures to confine carcinogens and a proposal for experiments to demonstrate confinement by using model noncarcinogens. Task 2 consisted of a proposal for the synthesis of model carcinogens and an experimental design for estimating the stability of carcinogens during storage.

Significance to Biomedical Research and the Program of the Institute: Studies of carcinogenic agents and the carcinogenesis process are important for several reasons, notably (1) because such studies will develop information that may lead to the prevention or, at least, reduction in incidence of certain forms of cancer and (2) because such studies will accumulate knowledge that will contribute to a better understanding of the nature and manifestation of cancer. The availability of well-characterized specimens of carcinogens and potential carcinogens is essential to research on chemical carcinogenesis to assure that the results are not influenced by contaminants.

Proposed Course: Compounds recently assigned by the NCI, as well as those that may be requested in the future, will be synthesized, purified, fully characterized, and sent to the Chemical Repository for distribution.

Date Contract Initiated: November 1, 1975

Current Annual Level: Since this is a Basic Ordering Agreement, the level of expenditures is assigned to each task as it is developed.

STANFORD RESEARCH INSTITUTE (NO1-CP-65741/BOA)

Title: Preparation of Carcinogens

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To synthesize, purify, and thoroughly characterize designated chemicals required for research on chemical carcinogenesis. This project is designed to serve a dual purpose: (1) to provide the Chemical Repository

of the Carcinogenesis Program with chemicals that are unavailable from other sources; (2) to provide well-characterized reference samples to investigators, through the Repository, to eliminate variability in experimental results that might be caused by impurities in inadequately analyzed specimens from various sources.

Major Findings: No major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: It is estimated that 80 to 90 percent of the cancers that occur in man are caused by carcinogenic substances in the environment. By studying these substances and their mechanisms of action, information may be derived that will lead to means of prevention of cancer in man.

Proposed Course: At the present time, 1 kg of 4-hydroxybutyl butyl nitrosamine is being synthesized for distribution by the Chemical Repository.

Date Contract Initiated: November 26, 1975

Current Annual Level: Since this ia a Basic Ordering Agreement, the level of expenditure is assigned to each new task as it is developed.

STANFORD RESEARCH INSTITUTE (NO1-CP-55672/BOA)

Title: Preparation of Various N-Nitroso Compounds

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. Larry Keefer

Objectives: To synthesize adequate quantities of isotopically-labelled N-nitrosamines that have been requested for investigations of the biological action of these compounds in initiating cancer. In addition, larger quantities of unlabelled N-nitrosamines have been prepared for reference purposes and for supplying material for studies on the carcinogenicity of these compounds.

Major Findings: A number of N-nitroso derivatives of secondary amines have been prepared for studies in carcinogenesis during the past year. They include: 1 kg of 4-hydroxybutylbutylnitrosamine, 200 g of methyl-N-benzylnitrosamine, 100 g of N-nitrosopyrrolidine, 200 g of N-nitrosomorpholine, 200 g of N-nitroso-sarcosine, 200 g of N-ntirosoproline, 200 g of N-nitroso-4-hydroxy proline, 100 g of N-ethyl-N'-nitro-N-nitrosoguanidine, 20 mCi ^{14}C-labelled N-(^{14}C-methyl)-acetoxymethyl nitrosamine, 11 mCi N-(^{14}C-methyl)-nitrosourethane, and 6 mCi N-(^{14}C-methyl)methoxymethylnitrosamine.

Significance to Biomedical Research and the Program of the Institute: Nitrosamines are known to cause cancer. The nature and location of the tumor depends on the specific nitrosamine. Much work is going on throughout the world to understand the mechanism of carcinogenesis. The nitrosamines offer special opportunities to study the mechanism of carcinogenesis because of this specificity. The preparation of radiolabelled nitrosamines will be useful for such research.

Proposed Course: A number of additional nitrosamines have been requested by NCI and will be synthesized during the coming year. They include: 200 g quantities of methyl ethyl nitrosamine, butyl nitrosourea, methyl-t-butyl-nitrosamine, methyl acetoxymethylnitrosamine, and N-nitroso nornicotine. In addition, 2 mCi of methane diazotate *cis*-conformer are to be prepared.

Date Contract Initiated: February 1, 1975

Current Annual Level: Since this is a Basic Ordering Agreement, the level of expenditures is assigned to each task as it is developed.

THERMO ELECTRON CORPORATION (NO1-CP-65822)

Title: Production and Delivery of Three HPLC Interface Systems

Contractor's Project Director: Dr. David H. Fine

Project Officer (NCI): Dr. Larry Keefer

Objectives: Update three TEA systems to include capability of interfacing with high pressure liquid chromatography (HPLC).

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Assist in detection of N-nitroso compounds in the environment.

Proposed Course: Project completed March 31, 1977.

Date Contract Initiated: September 30, 1976

Current Annual Level: $29,182

UTAH, UNIVERSITY OF (NO1-CP-45600)

Title: Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis

Contractor's Project Director: Dr. Ernst J. Eichwald

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) To determine the effects of chronic treatment with methotrexate, ALG, cyclophosphomide or cortisone acetate on benz(a)pyrene and ultraviolet-induced carcinogenesis in mice, and (2) to investigate various aspects of UV-induced carcinogenesis and UV-induced selective immunosuppression in mice.

Major Findings: (1) The dosage and regime for the drugs methotrexate, ALG, cyclophosphomide and cortisone acetate which permit chronic administration

without excessive morbidity have been determined. (2) The dosage and regime for both benz(a)pyrene and ultraviolet light resulting in an approximate 50% tumor incidence have been established. (3) Eight individual large-scale experiments were initiated approximately 16 months ago to investigate the combined effect of the immunosuppressants and carcinogens. At this time, 6 of the 8 studies have been completed and the data from these are currently being statistically evaluated. The remaining two studies will probably be completed within 6 months. (4) The interesting characteristics exhibited by the majority of skin tumors induced with UV (280-310 nm) light have been investigated further. Studies into this system have established: (a) the majority of UV tumors are highly antigenic and rejected by normal syngeneic recipients; (b) chemical immunosuppression renders normal mice susceptible to tumor challenge; (c) short-term UV exposure (subcarcinogenic) is also capable of rendering normal mice susceptible to UV tumor challenge; (d) the immune system of UV exposed mice appears to function normally in a number of different assay systems, suggesting that UV is not a general immunosuppressant; (e) adoptive transfer experiments have established that UV exposure causes an elevation in suppressor T-cell activity. This enhancement allows the antigenic UV-tumors to progress; (f) primary and secondary in vitro sensitization responses have established that spleen cells from normal, UV exposed, tumor bearing, and tumor immune mice are all capable of expressing cytotoxic lymphocyte activity. The lymphocytes from UV-exposed and tumor-bearing mice react with secondary type kinetics, suggesting a prior exposure to tumor-associated antigens or antigens crossreactive with them.

Significance to Biomedical Research and the Program of the Institute: This study is directed toward the following questions: Is the current trend toward increasing clinical use of immunosuppressive drugs likely to elevate the incidence of human malignant disease? Can the effects of these drugs on carcinogenesis be directly attributed to their immunosuppressive activity? Attempts are being made to provide information related to these questions through the use of carefully defined animal model systems. In addition, findings on the antigenicity of UV-induced tumors provide a unique opportunity to investigate the mechanism which enables highly antigenic tumors to escape immunologic destruction in the primary host. This mechanism appears to involve the presence of suppressor T-lymphocytes.

Proposed Course: (1) To finish the two outstanding experiments. (2) To subject all data collected to statistical evaluation.

Date Contract Initiated: May 7, 1971

Current Annual Level: $138,654

6. PUBLICATIONS OF THE CARCINOGENESIS PROGRAM

A. INTRAMURAL PROGRAM[1]

A1. Bast, R.C., Jr., Okuda, T., Plotkin, E., Tarone, R., Rapp, H.J., and Gelboin, H.V.: Development of an assay for aryl hydrocarbon (benzo[a]pyrene) hydroxylase in human peripheral blood monocytes. Cancer Res. 36: 1967-1974, 1976.

A2. Basu, M., Basu, S., Shanabruch, W.G., Moskal, J.R., and Evans, C.H.: Lectin and cholera toxin binding to guinea pig tumor (104Cl) cell surfaces before and after glycospingolipid incorporation. Biochem. Biophys. Res. Commun. 71: 385-392, 1976.

A3. Bates, R.R.: Laboratory approaches to the identification of carcinogens. Ann. N.Y. Acad. Sci. 271: 29-38, 1976.

A4. Buehring, Y.S., Poirier, L.A. and Stokstad, E.L.R.: Folate deficiency in the livers of diethylnitrosamine-treated rats. Cancer Res. 36: 2775-2779, 1976.

A5. Burreson, B.J., Moore, R.E. and Roller, P.P.: Volatile halogen compounds in the alga Asparagopsis taxiformis (Rhodophyta). J. Agric. Food Chem. 24: 856-861, 1976.

A6. Chen, B.P., Kung, H.F., and Bates, R.R.: Effects of methylation of the beta-galactosidase genome upon in vitro synthesis of beta-galactosidase. Chem. Biol. Interact. 14: 101-111, 1976.

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A10. Day, R.S., III.: UV-induced alleviation of K-specific restriction of bacteriophage λ. J. Virol. (In Press).

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[1]Papers published exclusively from the Intramural Program's staff.

A11. Day, R.S., III, and DiMattina, M.: Photodynamic action of chlorpromazine on adenovirus 5: Repairable damage and single-strand breaks. Chem. Biol. Interact. (In Press).

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A14. De Luca, L.M.: Review on mode of action of vitamin A in epithelial tissues. Vitamin (Japan) 50: 345-349, 1976.

A15. De Luca, L.M.: The molecular involvement of vitamin A in glycosyl transfer reactions of mammalian membranes. In Vitam. Horm. (In Press).

A16. De Luca, L.M.: Vitamin A. In De Luca, H.F. (Ed.): Fat-Soluble Vitamins. (In Press).

A17. De Luca, L.M., Frot-Coutaz, J.P., Silverman-Jones, C.S., and Roller, P.P.: Chemical synthesis of phosphorylated retinoids: Their mannosyl acceptor activity in rat liver membranes. J. Biol. Chem. 252: 2574-2579, 1977.

A18. Dingman, C.W. and Kakunaga, T.: DNA strand breaking and rejoining in response to ultraviolet light in normal human and xeroderma pigmentosum cells. Int. J. Radiat. Biol. 30: 55-66, 1976.

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A27. Edwards, W.D. and Bates, R.R.: Transplantation of isologous prostatic tissue in cleared mammary fat pads of Balb/c mice and F_{344} rats. Transplantation 20: 303-307, 1976.

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B. COLLABORATIVE PROGRAM[1]

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Pyeatte, J. - C4

Quadros, E. - A63

Rabin, E. - A30
Raha, C. - B481
Rainy, W. - B482
Rajewsky, M. - B483
Rao, M. - B484, B485, B486, B487, B488, B550
Rapp, H. - A1, A37, A38, A72, A142
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Regan, J. - B44, B45, B46, B73, B496, B497, B498, B499, B500, B501, B502, B551, B643, B644, B690
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Reuber, M. - B128
Revel, M. - B76, B478, B693
Reynafarje, B. - B58, B59, B60, B61, B503
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Ribi, E. - A142
Ricard, M. - B510
Rice, J. - A61, A65, A66, A85
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Rijnberk, A. - B332
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Rosenberg, A. - B199
Rosenkranz, H. - B387, B518, B519, B579, B676
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Ross, R. - B147, B150
Rossi, A. - B363
Roth, R. - B83, B84, B85
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Rozengurt, E. - B521, B522
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Schiller, A. - B332
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Seeger, R. - B539
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Sela, I. - B527
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Shih, T. - B562
Shils, M. - B160
Shimizu, H. - B626, B630
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Soong, L. - B199
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Speier, J. - B646, B647, B648
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Walker, A. - B530
Walker, C. - B668
Wallcave, L. - B175, B176, B177, B419
Wang, C. - B633, B634, B635
Ward, E. - B448
Warren, B. - B568, B636
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Weisman, M. - B607
Weiss, L. - B663
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Wepsic, H. - B664
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Wetzel, B. - A74
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Wigler, M. - B655, B656, B657, B665, B666
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Wiley, J. - A69
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Wilkoff, L. - B95, B96, B97, B98, B669
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Williams, G. - B671, B672, B673, B688, B689
Williams, M. - B430
Wilson, C. - B404
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Wilson, R. - B5, B9, B230, B636
Winocour, E. - B178, B444, B478, B674
Wishnok, J. - B13, B14, B605, B606, B675
Wislocki, P. - B676
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Yunis, E. - B145
Yuspa, S. - A28, A29, A138, A139, A140, A141, C46, C58

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Zbar, B. - A142
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7. APPENDIX - CHEMICALS BEING TESTED FOR CARCINOGENICITY

CHEMICALS FOR WHICH FINAL REPORTS ARE AVAILABLE TERMINAL SACRIFICE SCHEDULED BEFORE JULY 15, 1976

NUMBER	CHEMICAL	REPORT DATE
C02766	Nitrilotriacetic Acid	4/77
C01445	Nitrilotriacetic Acid Trisodium Salt Monohydrate	4/77
C00099	Chlordane	4/77
C00191	Chlordecone (Kepone)	1/76
C02686	Chloroform	6/76
C00135	Dimethoate	3/77
C04626	1,1,1-Trichloroethane	3/77
C04546	Trichloroethylene	1/76
C00180	Heptachlor	4/77
C01741	Phenformin	3/77

CHEMICALS FOR WHICH REPORTS HAVE BEEN DRAFTED

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C03974	Acetic Acid, (Ethylenedinitrilo) tetra trisodium salt (EDTA)	4/77	7/77
C04137	Acridine, 3,6-diamino-, HCL (Proflavin)	3/77	7/77
C00044	Aldrin	7/77	10/77
C01821	Aniline, N,N-dimethyl-p-nitroso-	6/77	10/77
C01923	Anthraquinone, 2-methyl-1-nitro	3/77	9/77
C02697	Aspirin, Phenacetin, Caffeine	7/77	11/77
C00066	Azinphos-methyl	7/77	10/77
C00419	Benzene, pentachloronitro-	6/77	12/77

CHEMICALS FOR WHICH REPORTS HAVE BEEN DRAFTED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C02664	Biphenyl, chloro-	10/77	1/78
C00077	Captan	7/77	10/77
C00544	Mexacarbate	7/77	10/77
C00055	Chloramben	7/77	10/77
C00408	Chlorobenzilate	5/77	11/77
C00533	Chloropicrin	6/77	11/77
C00102	Daconil (Chlorothanolil)	6/77	9/77
C01718	Dapsone	8/77	10/77
C03269	Diarylanilide Yellow	8/77	11/77
C00113	Dichlorovos (Vapona)	5/77	8/77
C00124	Dieldrin	6/77	10/77
C01547	IPD	5/77	10/77
C00566	Endosulfan	6/77	11/77
C00157	Endrin	3/77	9/77
C04535	Ethane, 1,1-dichloro-	5/77	10/77
C00511	Ethane, 1,2-dichloro-	7/77	10/77
C03554	Ethane, 1,1,2,2-tetrachloro-	6/77	9/77
C04579	Ethane, 1,1,2-trichloro-	7/77	11/77
C04580	Ethylene, tetrachloro-	7/77	9/77
C02653	Hexachlorophene	6/77	10/77
C01638	Isophosphamide	6/77	10/77
C01478	Lasiocarpine	6/77	10/77
C00204	Lindane	5/77	9/77

CHEMICALS FOR WHICH REPORTS HAVE BEEN DRAFTED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C00215	Malathion	6/77	10/77
C00420	Nitrofen	6/77	9/77
C00237	Picloram	7/77	10/77
C00226	Parathion	8/77	11/77
C00588	Phosphamidon	6/77	9/77
C01810	Procarbazine hydrochloride	6/77	10/77
C00500	Propane, 1,2-dibromo-3-chloro	6/77	10/77
C04615	Propene, 3-chloro- (allyl chloride)	6/77	11/77
C00168	Tetrachlorvinphos	6/77	10/77
C01865	Toluene, 2,4-dinitro-	6/77	9/77
C00259	Toxaphene	6/77	10/77
C03270	Tris (2,3-dibromopropyl) phosphate	6/77	11/77
C00599	Photodieldrin	6/77	9/77

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C01967	Acenaphthene, 5-nitro-	8/77	11/77
C02108	Acetamide	1/78	4/78
C01887	Acetanilide, 3-amino-4-ethoxy	12/77	3/78
C01978	P-acetophenetide, 3'-nitro-	11/77	2/78
C01536	Acronycine	9/77	12/77
C04682	Actinomycin D	1/78	4/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C02095	Adipamide	12/77	3/78
C02437	Aminochlorodiphenyl ether	12/77	3/78
C03736	Aniline	12/77	3/78
C02039	Aniline, p-chloro-	10/77	1/78
C02255	Aniline, 2,4-dimethoxy-, HCL	11/77	2/78
C01990	Aniline, 4,4'methylene-bis(N,N'dimethyl)	11/77	2/78
C01707	Aniline, 4,4'thiodi-	9/77	12/77
C02460	Aniline, 2,4,6-trichloro	12/77	3/78
C03587	Aniline, 2,4,5-trimethyl-, HCL	12/77	3/78
C02288	Aniline, 2,4,6-trimethyl-, HCL	12/77	3/78
C02993	P-anisidine, 2-methyl-	9/77	12/77
C01934	O-aniside, 5-nitro-	8/77	11/77
C03758	P-anisidine hydrochloride	1/78	4/78
C01989	Anisole, 2,4-diamino-, sulphate	11/77	2/78
C03032	Anisole, 2,3,5,6-tetrachloro-4-nitro-	1/78	4/78
C01730	Anthranilic acid	10/77	1/78
C01945	Anthranilic acid, 4-nitro-	8/77	11/77
C01876	Anthraquinone, 2-amino-	8/77	11/77
C01901	Anthraquinone, 1-amino-2-methyl	8/77	11/77
C04728	Arabinosylcytosine	1/78	4/78
C02120	L-arginine glutamate	12/77	3/78
C01569	5-azacytidine	7/77	10/77
C03474	Azathioprine	1/78	4/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C06371	Aziridine, 2-methyl	7/77	10/77
C02926	Azobenzene	3/78	6/78
C02426	Benzene, 1-chloro-2,4-dinitro-	12/77	3/78
C02415	Benzene, 1-chloro-2-nitro	12/77	3/78
C02404	Benzene, 1-chloro-4-nitro-	12/77	3/78
C01912	Benzimidazole, 6-nitro-	11/77	2/78
C02006	Benzophenone, 4,4'bis(dimethylamino)-	11/77	2/78
C03850	P-benzoquinone, dioxime	11/77	2/78
C06382	Bis(2-hydroxyethyl)dithiocarbamic acid, potassium salt	7/77	10/77
C01592	1,4-butanediol, dimethanesulfonate	9/77	12/77
C01434	1-butanol, 4-(butylnitrosamino)-	7/78	10/78
C02131	N-butylurea	11/77	2/78
C02664	Arochlor 1254	6/77	10/77
C04808	Carbanilide, 4,4'diacetyl-bis(amidino-hydrazone), dimethane sulfonate	1/78	4/78
C01898	Carbazole, 3-amino-9-ethyl-	11/77	2/78
C03043	Carbazole, 3-amino-9-ethyl-, HCL	11/77	2/78
C04591	Carbon disulfide	12/77	3/78
C06417	Carbonic acid, cyclic ethylene ester	7/77	10/77
C03485	Chlorambucil	1/78	4/78
C01558	Cholesterol, (P-(bis(2-chloroethyl) amino)phenyl) acetate	12/77	3/78
C02982	P-cresidine	10/77	1/78
C02277	Cyclamate, Sodium salt	12/77	3/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C04900	Cyclophosphamide	1/78	4/78
C01683	Daraprim	1/78	4/78
C04693	Daunomycin	1/78	4/78
C04933	O,P'-DDD	1/78	4/78
C00475	P,P'-DDD	7/77	10/77
C00555	P,P'-DDE	7/77	10/77
C00464	P,P'-DDT	7/77	10/77
C01581	Beta-deoxythioguanosine	10/77	1/78
C06393	Diallate	7/77	10/77
C03656	Dibenzo-p-dioxin	1/78	4/78
C03667	Dibenzo-p-dioxin, 2,7-dichloro	1/78	4/78
C03678	Dibenzo-p-dioxin, 1,2,3,4,6,7,8,9-Octac	1/78	4/78
C01661	Dibenzyline hydrochloride	10/77	1/78
C04795	Dibromodulcitol	1/78	4/78
C04762	Dibromomannitol	1/78	4/78
C02028	Dibutyltin diacetate	12/77	3/78
C00486	Dicofol (kelthane)	8/77	11/77
C02448	Dicyclopentadiene dioxide	12/77	3/78
C03689	P-dioxane	1/78	4/78
C00395	Dioxathion	7/77	10/77
C02700	Dipentylamine, N-nitroso-	3/78	6/78
C02244	Diphenylamine, 4-nitroso-	9/77	12/77
C03009	2,5-dithiobiurea	12/77	3/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C03918	DMBA	3/78	6/78
C01605	Emetine	9/77	12/77
C01570	Estradiol mustard	12/77	3/78
C00522	Ethane, 1,2-dibromo	8/77	11/77
C04604	Ethane, Hexachloro	8/77	11/77
C06406	Ether, bis(2-chloroethyl)-	7/77	10/77
C01694	Ethionamide	10/77	1/78
C03281	Ethylenediamine, n-1-naphthyl-, dihydrochloride	10/77	1/78
C05710	Griseofulvin	3/78	6/78
C01423	Guanidine, 1-methyl-3-nitro-1-nitroso-	7/78	10/78
C02142	Hexanamide	12/77	3/78
C03054	Hydantoin, dichlorodimethyl-	8/77	11/77
C04739	Hydrazine, 1-acetyl-2-picolinoyl	1/78	4/78
C04819	Guanazole	1/78	4/78
C01854	Hydrazine, 1,1-diphenyl	8/77	11/77
C03258	Hydroxylamine, n-nitroso-n-phenyl-ammonium salt	9/77	12/77
C04831	Hydroxyurea	1/78	4/78
C04717	Imidazole-4-carboxamide-5-(3,3-dimethyl-1-triazeno)-	1/78	4/78
C01616	Imidazole mustard	1/78	4/78
C02175	Isocyanic acid, 3,3'-dimethoxy-4,4'-biphenylene ester	9/77	12/77
C01489	Lead acetate	3/78	6/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C04853	Melphalan	1/78	4/78
C04944	O-melphalan	1/78	4/78
C04886	6-mercaptopurine	1/78	4/78
C04637	Methane, trichlorofluoro-	12/77	3/78
C04568	Methane, triiodo- (Iodoform)	7/77	10/77
C03190	Methansulfon-M-aniside,4'-(9-acridinyl-amino)-, monohydrochloride	3/77	6/78
C03849	Methiodal Sodium	10/77	1/78
C04671	Methotrexate	1/78	4/78
C04875	Methotrexate, dichloro-	1/78	4/78
C00497	Methoxychlor	7/77	10/77
C02380	4,4'-methylene-bis-(2-chloroaniline) hydrochloride	12/77	3/78
C03010	Methyl orange B	12/77	3/78
C04784	Methylthioinosine	1/78	4/78
C06428	Mirex	7/77	10/77
C04706	Mitomycin C	1/78	4/78
C03021	1,5-naphthalenediamine	10/77	1/78
C01956	Naphthalene, 1-nitro-	9/77	12/77
C00431	Niclosamid	11/77	2/78
C03792	Nithiazide	1/78	4/78
C04740	1-nitrosourea, 1-(2-chloroethyl)-3-cycl	1/78	4/78
C02084	Nitrous acid, sodium salt	1/77	2/78
C06439	Oxamide, dithio-	7/77	10/77

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C04126	2-oxetanone, 3,3-dimethyl	12/77	3/78
C03963	Phenol, 4-amino-2-nitro	12/77	3/78
C03305	M-phenylenediamine, 4-chloro-	12/77	3/78
C03292	O-phenylenediamine, 4-chloro-	6/77	9/77
C02482	M-phenylenediamine, dihydrochloride	12/77	3/78
C02493	O-phenylenediamine, dihydrochloride	12/77	3/78
C03941	O-phenylenediamine, 4-nitro-	2/78	5/78
C02222	P-phenylenediamine, 2-nitro-	12/77	3/78
C02233	P-phenylenediamine, n-phenyl-, HCL	12/77	3/78
C03316	P-phenylenediamine sulfate, 2-chloro-	12/77	3/78
C04922	Phosophorodiamidic acid, N,N-bis(2-chloroethyl)-N'-3-hydroxypropyl cyclohexylamine	1/78	4/78
C01627	2,6-piperazinedione-4,4'propylene dioxo-piperazine	1/78	4/78
C02813	Piperonyl butoxide	3/78	6/78
C02824	Piperonyl sulfoxide	3/78	6/78
C03350	Propane Sultone	7/77	10/77
C06440	1,2-Propanediol, 3-chloro-	7/77	10/77
C03076	Propionic acid, 3-nitro-	8/77	11/77
C01785	Pyrazinecarboxamide	11/77	2/78
C01672	Pyridium	12/77	3/78
C02506	Saccharine	7/77	10/77
C06097	Saccharine, sodium salt	12/77	3/78
C05981	Semicarbazide, monohydrochloride	7/77	10/77

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C06462	Sodium azide	7/77	10/77
C06473	Sodium Bisulfide	7/77	10/77
C02459	4'-stilbenamine, 2,5-dimethoxy	12/77	3/78
C03167	Streptozotocin	1/78	4/78
C02200	Styrene	11/77	2/78
C02211	Styrene, beta-nitro-	12/77	3/78
C03827	Succinic acid, mono(2,2-dimethylhydrazi)	12/77	3/78
C00453	Sulfallate (CDEC)	9/77	12/77
C02379	3,3'4,4'-tetraaminodiphenyl tetrahydro-chloride	12/77	3/78
C02471	Tetrafluoro-m-phenylenediamine dihydro-chloride	12/77	3/78
C03065	Thiazole, 2-amino-5-nitro-	8/77	11/77
C04897	Prednisone	1/78	4/78
C04557	Thiophene, 2,5-dihydro-, 1,1-dioxide-	9/77	12/77
C06451	Thiosemicarbazone	7/77	10/77
C01649	Thio tepa	10/77	1/78
C01832	Toluene-2,5-diamine, sulfate	7/77	10/77
C05129	Toluene, 2,4-diamino-, hydrochloride	12/77	3/78
C02346	M-toluidine hydrochloride	12/77	3/78
C02040	P-toluidine, 3-chloro	1/78	4/78
C02051	O-toluidine, 5-chloro	1/78	4/78
C02357	P-toluidine, hydrochloride	12/77	3/78
C01843	O-toluidine, 5-nitro-	7/77	10/77

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C02153	P-tolylurea	12/77	3/78
C02391	S-triazine, 2,4-diamino-6-phenyl-	12/77	3/78
C00442	Trifluralin	7/77	10/77
C00260	Triphenyltinhydroxide	1/78	4/78
C01729	Tryptophan, L-	11/77	2/78
C01490	Uracil, 2-thio	3/78	6/78
C04820	Uracil mustard	1/78	4/78
C02119	Urea	12/77	3/78
C03247	Urea, 1-((p-acetylphenyl)sulfonyl)-3-cyclohexyl	11/77	2/78
C04773	Urea, 1,3-bis(2-chloroethyl)-1-nitroso-	1/78	4/78
C03085	Urea, (2-bromo-2-ethylbutyryl)-	10/77	1/78
C01763	Urea, 1-butyl-3-(p-tolylsulfonyl)-	10/77	1/78
C01752	Urea, 1-((P-chlorophenyl)sulfonyl)-3-PR	11/77	2/78
C04524	Urea, N,N'-dicyclohexylthio	9/77	12/77
C03816	Urea, 1,3-diethyl-2-thio-	12/77	3/78
C02073	Urea, (p-ethoxyphenyl)-	12/77	3/78
C03372	Urea, ethylenethio-	7/77	10/77
C03327	Urea, 1-(hexahydro-1H-azepin-1-YL)-3-p-tolysulfonyl)-	11/77	2/78
C02017	Urea, 1-phenyl-2-thio-	12/77	3/78
C02186	Urea, 1,1,3-trimethyl-2-thio-	10/77	1/78
C04842	Vinblastine	1/78	4/78
C04864	Vincristine	1/78	4/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C06484	Vinylene carbonate	7/77	10/77
C02313	2,4-xylidine hydrochloride	12/77	3/78
C02324	2,5-xylidine hydrochloride	12/77	3/78
C03770	Acetanilide, 4'-(chloroacetyl)-	11/77	2/78
C02960	Ammonium, (2-chloroethyl)trimethyl-, CH	3/78	6/78
C02299	Aniline, 2,4,5-Trimethyl	3/78	6/78
C03747	O-anisidine hydrochloride	2/78	5/78
C03521	1H-benzotriazole	1/78	4/78
C02937	Calcium Carbimide	3/78	6/78
C03565	C.I. vat yellow 4	3/78	6/78
C03861	5 Beta-cholan-24-oic acid, 3 alpha-hydrox	11/77	2/78
C03598	P-cresol, 2,6-di-tert-butyl-	3/78	6/78
C02880	Diphenylamine, n-nitroso-	3/78	6/78
C02868	Ethane, 1,1-dichloro-2,2-bis(p-ethylphe)	3/78	6/78
C02857	Ethyl tellurac	3/78	6/78
C02959	Ethyl tuads	3/78	6/78
C02891	Lead dimethyldithiocarbamate	3/78	6/78
C03952	Norphenazone	12/77	3/78
C02904	Phenol, 2,4,6-trichloro-	3/78	6/78
C03930	P-phenylenediamine, dihydrochloride	12/77	3/78
C02971	Phosphorothioic acid, O,O-dimethyl O-(P-nitrophenyl) ester	3/78	6/78
C03612	Phthalamide	3/78	6/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C03601	Phthalic anhydride	3/78	6/78
C03907	Pyridine, 2-(chloromethyl)-, HCL	11/77	2/78
C03838	Pyridine, 3-(chloromethyl)-, HCL	11/77	2/78
C02835	Sodium dimethyldithiocarbamate	3/78	6/78
C02302	Toluene-2,4-diamine	3/78	6/78
C02368	O-toluidine, 4-chloro-, hydrochloride	12/77	3/78
C02335	O-toluidine hydrochloride	12/77	3/78
C03781	Trimethyl phosphate	10/77	1/78
C03963	4 amino-2-nitrophenol	1/78	4/78
C03827	2,2-dimethylhydrazide	1/78	4/78
C03838	3-pyridine HCL	1/78	4/78
C03781	Trimethylphosphate	1/78	4/78
C02233	N-phenyl-p-phenylenediamine	1/78	4/78
C02255	2,4-dimethoxyaniline HCL	2/78	5/78
C02175	3,3-dimethoxybenzidine	2/78	5/78
C03861	Lithocholic acid	2/78	5/78
C03850	P-quinone dioxime	2/78	5/78
C03849	Sodium iodomethane sulfonate	2/78	5/78
C03930	P-phenylenediamine DiHCL	2/78	5/78
C03816	N,N'-diethylthiourea	2/78	5/78
C02028	Dibutyctin diacetate	2/78	5/78
C04126	Pivalolactone	3/78	6/78
C00260	Triphenyltin hydroxide	3/78	6/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C03941	4-nitro-o-phenylenediamine	3/78	6/78
C02186	1,1,3-trimethyl-2-thiourea	3/78	6/78
C03952	1-phenyl-3-methyl-5-pyrazole	3/78	6/78
C03805	Bromo-ethylbutrylurea	3/78	6/78
C01990	4,4'methylenebis	6/78	9/78
C02017	1-phenyl-2-thiourea	6/78	9/78
C02200	Styrene	6/78	9/78
C02211	B-nitrostyrene	6/78	9/78
C02040	3-chloro-p-toluidine	8/78	11/78
C02051	5-chloro-o-toluidine	8/78	11/78
C00420	Nitrofen	8/78	11/78
C02244	P-nitrosodiphenylamine	8/78	11/78
C03907	2,-pyridine HCL	4/78	7/78
C03792	Nithiazide	4/78	7/78
C03770	4,4'-chloroacetyl	6/78	9/78
C02039	p-chloroaniline	6/78	9/78
C02222	2-nitro-p-phenylene diamine	6/78	9/78
C02006	4,4'-bis-benzophenone	3/78	6/78
C02813	Piperonyl butoxide	3/78	6/78
C03565	Amathrene	3/78	6/78
C02824	Piperonyl sulfoxide	3/78	6/78
C02891	Ledate	7/78	10/78
C02857	Ethyl tellurac	4/78	7/78

CHEMICALS FOR WHICH RESULTS ARE BEING VERIFIED (cont.)

NUMBER	CHEMICAL	DATE DRAFT EXPECTED	DATE REPORT EXPECTED
C03598	2,6-di-tert-butyl-p-cresol (BHT)	5/78	8/78
C02835	Sodium dimethyldithiocarbamate (SDDC)	9/78	12/78
C02335	O-toluidine hydrochloride	6/78	9/78
C02926	Azobenzene	5/78	8/78
C02868	Pertane	7/78	10/78
C02904	Omal	9/78	12/78
C02971	Methyl parathion	7/78	10/78
C03601	Phthalic anhydride	6/78	9/78
C02299	2,4,5-trimethyl aniline	6/78	9/78
C02960	2-chloroethyl trimethylammonium chloride	8/78	11/78
C03612	Phthalamide	8/78	11/78
C02937	Calcium cyanamide	8/78	11/78
C02302	2,4-toluene diamine	9/78	12/78
C02368	4-chloro-O-toluidine	4/78	7/78
C02959	Ethyl tuads	4/78	7/78
C02880	Redax	5/78	8/78

CHEMICALS BEING TESTED

NUMBER	CHEMICAL	DATE REPORT EXPECTED
C50215	Acrylonitrile	7/80
C01514	Adriamycin	2/78
C50475	Agar agar	7/80

NUMBER	CHEMICAL	DATE REPORT EXPECTED
C08640	Aldicarb	2/78
C50464	Allyl isothiocyanate	7/80
C50613	Amino undecanoic acid	7/80
C08684	Anilazine	2/78
C50011	Benzoin	5/78
C06360	Benzyl chloride	10/80
C50282	2-biphenylamine	10/79
C50635	Bisphenol A	7/80
C50602	1,3-butadiene	7/80
C54375	Butyl benzyl phthalate	7/80
C50646	Caprolactam	7/80
C53849	Carmoisine	11/79
C53781	C.I. Disperse yellow 3	7/80
C03510	Cinnamyl anthranilate	7/80
C08662	Coumaphos	1/78
C55005	Cyclohexanone	10/80
C50737	Cytembena	11/79
C53792	D & C red #9	7/80
C50657	Diallylphthalate	7/80
C50260	1,4-diamino-3,6-dichlorobenzene	7/80
C08673	Diazinon	4/78
C03703	Dibenzo-p-dioxin, 1,2,3,6,7,8-hexachloro-	11/78
C03714	Dibenzo-p-dioxin, 2,3,7,8-tetrachloro-	11/78

CHEMICALS BEING TESTED (cont.)

NUMBER	CHEMICAL	DATE REPORT EXPECTED
C03690	Dibenzo-p-dioxin, 2,3,7-trichloro-	11/79
C54262	1,1-dichloroethylene	7/80
C51581	Cis, 1,2-dichloroethylene	7/80
C54591	Trans, 1,2-dichloroethylene	7/80
C54386	Di(2-ethylhexyl) adipate	7/80
C52733	Di(2-ethylhexyl) phthalate	7/80
C50055	Dimethylterephthalate	4/78
C50668	4,4'-diphenylmethane diisocyanate	7/80
C54557	Direct black 38	7/80
C54579	Direct blue 6	7/80
C54568	Direct brown 95	7/80
C07001	Alpha-epichlorohydrin	7/80
C50135	Ethylene chlorohydrin	7/80
C00522	Ethane, 1,2-dibromo-	11/79
C50044	Ether, bis(2-chloro-1-methylethyl)-	8/78
C50384	Ethyl acrylate	7/80
C50453	Eugenol	7/80
C08651	Fenthion	2/78
C08695	Fluometuron	12/78
C50748	Gum arabic	7/80
C50395	Gum guar	7/80
C54364	Gum tara	7/80
C50259	Hexamethylmelamine	3/78

NUMBER	CHEMICAL	DATE REPORT EXPECTED
C05721	1,3-hexanediol, 2-ethyl-	7/80
C50419	Locust bean gum	7/80
C00215	Malathion	3/78
C50362	Mannitol	7/80
C08628	Malathion-o-analog	8/78
C50715	Melamine	7/80
C50000	Menthol	1/78
C50102	Methylene chloride	7/80
C50680	Methyl methacrylate	7/80
C54604	4,4'-methylenedianiline	7/80
C53838	Orange G (C.I. acid orange 10)	11/79
C50146	4,4'-oxydianiline	9/79
C53894	Pentachloroethane	7/80
C04580	Perchloroethylene	7/80
C50124	Phenol	2/80
C00500	Propane, 1,2-dibromo-3-chloro-	11/78
C50588	Propyl gallate	7/80
C50599	Propylene oxide	7/80
C50157	Reserpine	8/78
C50033	Selenium sulfide	7/80
C54546	Selsun	2/79
C53907	Sunset yellow FCF	11/79
C02722	Stannous chloride	7/80

CHEMICALS BEING TESTED (cont.)

NUMBER	CHEMICAL	DATE REPORT EXPECTED
C54977	Styrene oxide	10/80
C53929	Sudan I (solvent yellow 14)	7/80
C50022	Sulfisonazole	3/78
C52459	1,1,1,2-tetrachloroethane	7/80
C04240	Titanium oxide (TI 02)	1/78
C50317	Toluene-2,6-diamine dihydrochloride	7/79
C50533	2,4-toluene diisocyanate	7/80
C50373	Vinyl bromide	7/80
C50226	Zearalenone	7/80
C50442	Ziram	7/80

FRONT LOG SUMMARY

TERMINAL SACRIFICE SCHEDULED AFTER JULY 15, 1976

LAB	NUMBER	ROUTE	NAME	ALL IADR AT CBDS	EST. REPORT DATE
M	C03510	DF	CINNAMYL ANTHRANILATE	03/21/77	09/21/77
G	C08662	DF	COUMAPHOS	04/29/77	10/29/77
H	C50000	DF A	DL-MENTHOL	06/24/77	12/24/77
H	C04240	DF A	TITANIUM DIOXIDE	06/24/77	12/24/77
T	C03714	GAV	TCDD	04/29/77	10/29/77
T	C03703	GAV	HCDD	04/29/77	10/29/77
G	C08684	DF	ANILIZIDE	05/27/77	11/27/77
G	C08651	DF	FENTHION	05/27/77	11/27/77
G	C08640	DF	ALDICARB	05/27/77	11/27/77
H	C50022	GAV B	SULFISOXAZOLE	08/19/77	02/19/78
H	C50033	GAV B	SELENIUM DISULFIDE	08/19/77	02/19/78
S	C50259	IP/IJ	HEXAMETHYLMELAMINE	06/27/77	12/27/77
G	C08673	DF	DIAZINON	07/29/77	01/29/78
H	C50055	DF	DIMETHYL TEREPHTHALATE	07/29/77	01/29/78
H	C50011	DF	BENZOIN	08/26/77	02/26/78
G	C08628	DF	MALAOXON	11/28/77	05/28/78
G	C00215	DF	MALATHION	11/28/77	05/28/78
H	C50044	GAV	BCPE	11/28/77	05/28/78
S	C50157	DF	RESERPINE	01/27/78	07/27/78
T	C03714	SP A	TCDD	04/21/78	10/21/78
T	C03703	SP A	HCDD	04/21/78	10/21/78
G	C08695	DF	FLUOMETURON	03/31/78	09/30/78

FRONT LOG SUMMARY (cont.)

LAB	NUMBER	ROUTE	NAME	ALL IADR AT CBDS	EST. REPORT DATE
H	C50033	SP C	SELENIUM DISULFIDE	07/21/78	01/21/79
H	C54546	SP C	SELSUN	07/21/78	01/21/79
H	C50124	OW	PHENOL	07/28/78	01/28/79
M	C50317	DF	2,6-TOLUENEDIAMINE	10/27/78	04/27/79
H	C00522	INH D	EDB	01/19/79	07/19/79
H	C00500	INH D	DBCP	01/19/79	07/19/79
M	C50146	DF	4,4'-OXYDIANILINE	12/29/78	06/29/79
S	C01514	IP/IJ	ADRIAMYCIN	02/23/79	08/23/79
L	C50715	DF A	MELAMINE	06/22/79	12/22/79
L	C50613	DF A	AMINOUNDECANOIC ACID	06/22/79	12/22/79
L	C50260	DF A	1,4-DIAMINO-2,6-DICHLOROBENZENE	06/22/79	12/22/79
L	C50635	DF A	BISPHENOL A	06/22/79	12/22/79
M	C50282	DF	2-AMINOBIPHENYL	04/27/79	10/27/79
S	C50737	IP/IJ	CYTEMBENA	04/27/79	10/27/79
H	C50124	INH	PHENOL	05/25/79	11/25/79
L	C50657	GAV	DIALLYLPHTHALATE	05/25/79	11/25/79
B	C53849	DF A	RED #14	09/21/79	03/21/80
B	C53838	DF A	ORANGE #10	09/21/79	03/21/80
B	C53907	DF A	YELLOW #6	09/21/79	03/21/80
L	C50668	GAV B	4,4'DIPHENYLMETHANE DIISOCYANATE	09/21/79	03/21/80
L	C50646	GAV	CAPROLACTAM	07/27/79	01/27/80
M	C54375	DF A	BUTYLBENZYLPHTHALATE	09/21/79	03/21/80
M	C54386	DF A	DI-2-ETHYLHEXL-ADIPATE	09/21/79	03/21/80

FRONT LOG SUMMARY (cont.)

LAB	NUMBER	ROUTE	NAME	ALL IADR AT CBDS	EST. REPORT DATE
M	C52733	DF A	DI(2-ETHYLHEXL)PHTHALATE	09/21/79	03/21/80
S	C50453	DF A	EUGENOL	10/19/79	04/19/80
S	C50442	DF A	ZIRAM	10/19/79	04/19/80
S	C50362	DF A	MANNITOL	10/19/79	04/19/80
B	C53781	DF B	YELLOW #3	11/26/79	05/26/80
B	C53792	DF B	RED #9	11/26/79	05/26/80
B	C53929	DF B	YELLOW #14	11/26/79	05/26/80
G	C53894	GAV B	PENTACHLOROETHANE	11/26/79	05/26/80
G	C03554	GAV B	1,1,1,2-TETRACHLOROETHYLENE	11/26/79	05/26/80
G	C54262	GAV A	VINYLIDENE CHLORIDE	11/26/79	05/26/80
S	C50102	GAV A	METHYLENE CHLORIDE	11/26/79	05/26/80
M	C50419	DF B	LOCUST BEAN GUM	12/21/79	06/21/80
M	C50748	DF B	GUM ARABIC	12/21/79	06/21/80
M	C50395	DF B	GUM GUAR	12/21/79	06/21/80
S	C50304	GAV	ETHYL ACRYLATE	10/26/79	04/26/80
G	C04026	GAV	1,1,1-TRICHLOROETHANE	12/28/79	06/28/80
G	C51581	GAV C	CIS-1,2-DICHLOROETHYLENE	02/22/80	08/22/80
G	C54591	GAV C	TRANS-1,2-DICHLOROETHYLENE	02/22/80	08/22/80
D	C50099	INH A	PROPYLENE OXIDE	03/21/80	09/21/80
D	C50215	INH A	ACRYLONITRILE	03/21/80	09/21/80
D	C50680	INH A	METHYL METHACRYLATE	03/21/80	09/21/80
M	C54604	DF C	4,4'-METHYLENE DIANILINE	03/21/80	09/21/80
M	C54364	DF C	GUM TARA	03/21/80	09/21/80

LAB	NUMBER	ROUTE	NAME	ALL IADR AT CBDS	EST REPORT DATE
M	C50745	DF C	AGAR AGAR	03/21/80	09/21/80
S	C50464	GAV	ALLYL ISOTHIOCYANATE	01/25/80	07/25/80
G	C04580	GAV	TETRACHLOROETHYLENE	02/29/80	08/29/80
L	C50135	SP	ETHYLENE CHLOROHYDRIN	03/28/80	09/28/80
B	C54579	DF C	BLUE #6	06/20/80	12/20/80
B	C54557	DF C	BLACK #38	06/20/80	12/20/80
B	C54568	DF C	BROWN #95	06/20/80	12/20/80
D	C50602	INH A	1,3-BUTADIENE	06/20/80	12/20/80
D	C50077	INH B	PROPYLENE	06/20/80	12/20/80
D	C50373	INH B	VINYL BROMIDE	06/20/80	12/20/80
S	C50266	DF B	ZEARALENONE	06/20/80	12/20/80
S	C50588	DF B	PROPYL GALLATE	06/20/80	12/20/80
S	C02772	DF B	STANNOUS CHLORIDE	06/20/80	12/20/80
D	C07001	INH C	ALPHA-EPICHLOROHYDRIN	10/24/80	04/24/81
D	C04580	INH C	TETRACHLOROETHYLENE	10/24/80	04/24/81
D	C05050	INH C	DIMETHYLNITROSAMINE	10/24/80	04/24/81
S	C50168	GAV C	DEODORIZED COTTONSEED OIL	12/19/80	06/19/81
S	C54850	GAV C	NON-DEODORIZED COTTONSEED OIL	12/19/80	06/19/81
D	C50088	INH D	ETHYLENE OXIDE	02/23/81	08/23/81
D	C02799	INH D	FORMALDEHYDE	02/23/81	08/23/81
D	C50102	INH D	METHYLENE CHLORIDE	02/23/81	08/23/81

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